JOURNAL OF AGRICULTURAL RESEARCH

VOL. 73 WASHINGTON, D. C., OCTOBER 1 AND 15, 1946 Nos. 7 and 8

EFFECT OF TIME AND CONDITIONS OF CURE ON THE CARBOHYDRATE COMPOSITION OF SWEETPOTATOES AND THE PROPERTIES OF THEIR STARCHES [1]

By H. N. BARHAM, *industrial chemist*, and J. A. WAGONER, *assistant industrial chemist, Kansas Agricultural Experiment Station*

INTRODUCTION

The work herein reported began from an observation (*4*) [2] that the curing of sweetpotatoes (*Ipomoea batatas* (L.) Lam.) altered the quality of the starch which they contained. The similarity of sweetpotato starch to tapioca (*cassava*) starch and the industrial importance of the latter were regarded as adequate reasons for attempts at further modification by subjecting sweetpotatoes to curing conditions for periods longer than the usual 10 to 14 days.

Unaltered sweetpotato and tapioca starches are similar in respect to viscosity, differing prinicpally in that sweetpotato starch forms less gummy pastes and gelatinizes at a higher temperature. Both form clear pastes the physical characteristics of which change but slowly when left undisturbed at room temperature. The regular cure of sweetpotatoes lowers the extremes of the gelatinization temperature of the starch as well as the viscosity of its pastes, but not to the extent that the pasting curve approaches coincidence with that of tapioca starch. Paine and others (*12*), in summarizing recent work on the properties of sweetpotato starch, have emphasized the superior qualities which it possesses and have pointed out important uses in which it is capable of replacing tapioca starch. In view of the effects of cure on the pasting characteristics of sweetpotato starch, there was the possibility that control of enzymic changes in the tissue might not only make possible a further accentuation of the tapioca like quality of sweetpotato starch but that it might also be of value in interpreting the effects of storage and cure upon the quality of sweetpotato starch.

In setting up the experiments, arrangements for the accurate control of the conditions of cure were made with care, and interruptions were regarded as unlikely. Nevertheless, the control mechanism failed to operate properly on four different occasions during the course of the experiments, thereby permitting the temperature and humidity to fall. Subsequent evaluation of the data showed that the accidental lowering of the temperature and relative humidity was fortunate in that it provided reference points for variations in the carbohydrate composi-

[1] Received for publication December 22, 1944. Contribution No. 297, Department of Chemistry, Kansas Agricultural Experiment Station. Part of the material in this paper was taken from an unpublished thesis presented by J. A. Wagoner to the Graduate School of Kansas State College in partial fulfillment of the requirements for the degree of doctor of philosophy, 1943.

[2] Italic numbers in parentheses refer to Literature Cited, p. 279.

tion in the tissue, the ease of starch removal from the tissue, the amounts of two starch fractions removed from each sample, starch density, water-holding capacity of the granule, average granule diameter, materials adsorbed within the granules and removable by solvent extraction, alkali lability, and the rate of development of viscosity during pasting.

EXPERIMENTAL PROCEDURE

SWEETPOTATOES

Curing.—Twenty bushels of Improved Big Stem Jersey sweetpotatoes were dug from the farm of a local grower and delivered immediately to the laboratory. Five bushels were processed at once for the removal of starch, and the remaining 15 were placed in a room equipped to maintain a constant temperature and relative humidity. This room had been cleaned and fumigated, and the racks were so adjusted that the baskets of sweetpotatoes would have ample room for air circulation. The controls were set at 30° C. and for 90-percent relative humidity. However, because of the failure of the control mechanism to operate properly, the temperature and humidity dropped to 25° and 50-percent relative humidity, respectively, for 72 hours within the first week and for approximately 48 hours within the sixth-, twelfth-, and nineteenth-week periods. Weight records were made of each basket of sweetpotatoes as it was received and at the end of each week of cure. Sample baskets were removed at the end of the first, second, fourth, sixth, ninth, twelfth, sixteenth, and nineteenth week, and the sweetpotatoes were processed entirely for starch except for small amounts of tissue reserved for analyses.

Starch recovery.—Sample bushels of sweetpotatoes were taken from the constant temperature room and washed in cool water to remove adhering foreign matter. When clean and dry, they were ground three times in a meat grinder, during which time a small stream of a 0.15-percent sodium bisulfite solution was directed through the grinder to repress the enzymic action and to wash the grinder. Starch was removed from the pulp by kneading it through clean cotton bags until no more was obtainable. The starch was washed and settled three times in fresh 0.15-percent bisulfite solution after which it was tabled four times, dewatered on a Büchner funnel and placed on a clean protected surface to air-dry at room temperature. When the moisture content of the starch had dropped to a constant level at the prevailing humidity, it was stored for later examination. The above procedure gave the starch fraction which is designated as the A fraction. The B fraction was obtained from the washed pulp by regrinding it two additional times with a finer plate. In other respects, the procedure was the same. The B fractions were the result of efforts to increase yields; however, because of the possibility that the fractions might have different properties, they were examined separately. Even with the second grinding, total yields were low as compared with those obtained in commercial practice. The starches so obtained were white, odorless, and free from any foreign matter detectable under a magnification of 300 diameters.

Chemical analyses.—One-hundred-gram samples of pulped sweetpotatoes (to which no bisulfite had been added) were placed immedi-

ately after grinding in sufficient alcohol, allowing for the water content of the mash, to give a final concentration of 80 percent. The mixture was heated at 60° C. for 30 minutes and stored in sealed glass jars. When analyses were to be made, the solids were filtered off and washed first with warm 10-percent alcohol and then with warm distilled water. The solids were dried, ground to pass through a 200-mesh screen, and redried in vacuo at 100° to constant weight. Aliquot samples were analyzed for starch by direct acid hydrolysis according to official method XXVII, 30, of the Association of Official Agricultural Chemists (*1*). The acid hydrolysis method was selected because, in a number of respects, it has been found to give more satisfactory results (*2, 6, 11*).

The filtrate from each sample, together with the washings, were made to 500 cc. Since no turbidity developed when the water washings were added to the alcohol filtrates, it may be assumed that no dextrins were washed out of the alcohol-insoluble materials. However, had alcohol-soluble nonsugars been added to the filtrates, it is doubtful whether they would have been lost through clarification with neutral lead acetate (*9*). Analyses for reducing and total sugars were made by the A. O. A. C. official methods XXXIV, 37–41 (*1*).

Aliquot samples of the sugar solutions also were evaporated and dried according to the A. O. A. C. official method XXXIV, 5 (*1*). The solids present in the alcohol solutions added to the alcohol-insoluble solids represent the total solids.

STARCH

Moisture.—The starches were analyzed for moisture by heating weighed samples in a vacuum oven for 12 hours at a pressure of less than one inch of mercury and a temperature of 110° C. The loss in weight was taken to be equivalent to the amount of moisture in the starch.

Granule diameter.—Granule diameter measurements were made microscopically with a properly adjusted glycerine-starch suspension. Five hundred granules were measured in microns at a magnification of 300 diameters. Such measurements were found capable of duplication within 0.25 micron. The average granule diameter and granule-size distribution were computed for each starch.

The average granule diameter data obtained for the starches of the natural and solvent-extracted A series by the microscopic method were checked against those obtained by air-permeability measurements. The instrument used was the Fisher sub-sieve sizer which incorporates the design of Gooden and Smith (*8*). Measurements were made in accordance with the directions supplied by the manufacturer. The air-permeability method gave values which were consistently higher than those given by the microscopic method. However, the factors for the natural and solvent-extracted starches were different; they approximated 1.30 for the natural and 1.15 for the solvent-extracted starches. This difference was interpreted to mean that a change in the shape of the granules had occurred during solvent extraction.

Density.—Starch density was determined in pycnometers of roughly 25-cc. capacity and at a temperature of 25° C. The suspending liquids were benzene and water; 7 to 12 gm. of starch were used for each determination.

Solvent extraction.—The solvent extraction of starches was made with 85-percent methanol-water solutions according to the procedure of Schoch (*15*). The desorption of removable adsorbed materials was regarded as complete after five extractions. The solvent was completely removed at low temperature and pressure from the material extracted from the granules and the material was dried and weighed.

Protein content.—In order to ascertain the extent to which the material adsorbed within the granule was protein in character, starches were analyzed for nitrogen according to the Kjeldahl procedure. The A. O. A. C. official method, II, 21 (*1*) was adapted for use with 5 gm. of starch.

Alkali-labile values.[3]—These values were determined in accordance with the method developed by Taylor and others (*17*). Most starches were examined by the Schoch method (*16*). The latter values were smaller but roughly proportional to the Taylor values.

Viscosity records.—Viscosity measurements were made with a rotating-cylinder viscometer (*3*) which had been designed especially for the study of starch pastes. The procedure consisted of placing 40 gm. of starch, corrected for moisture, and 360 gm. of water, including that present in the starch, in the rotating cup, establishing a temperature of 20° C., heating at a rate of 0.5° per minute unti lthe temperature of the paste had reached 92°, cooking at this temperature for 30 minutes, and cooling to room temperature at the same rate that it was heated. Torque measurements, expressed in grams, were made with a frequency sufficient for making smooth curves.

EXPERIMENTAL RESULTS

In reporting the data accumulated in the course of these experiments it is recognized that the actual magnitude of each measurement depends upon the variety of sweetpotato selected and upon the environmental conditions under which the variety is grown. Moreover, there is the variability in granule structure which comes from enzymic changes in the tissue. Since the greatest value of the data is in indicating trends in changes due to time and conditions of cure, it is advantageous to present most data graphically; for economy of space, tables are given in those cases where there would be no sacrifice in clarity.

When curves are used to follow changes within the sweetpotato tissue, two curves, one a broken line, the other a solid line, are used to represent variations within each set of data. The broken-line curve is drawn through points of constant temperature and humidity; the solid-line curve is drawn through all points. The broken-line curves are not projected beyond the sixteenth week since there were no data subsequent to the nineteenth week corresponding to the reestablishment of the normal conditions of cure. Open circles are used to indicate points at which normal conditions of cure prevailed, closed circles to indicate points at which the temperature and relative humidity fell. In the following presentation of results, it is assumed that the broken-line curves approximate the effects of cure which could reasonably be expected had there been no interruption of

[3] Alkali-labile measurements were made by Carol L. Campbell of this laboratory.

temperature and humidity control. The solid-line curves reflect the effects of an accidental lowering of the temperature and humidity during cure which occurred within the first, sixth, twelfth, and nineteenth weeks.

SWEETPOTATO TISSUE

During the 19-weeks' cure, the sweetpotatoes changed considerably in appearance. Originally they were firm, crisp, yellow in color, and had a density greater than water, but at the end of 19 weeks, they were brown in color, woody in texture, wrinkled, and had a density less than water. Changes with respect to these factors were first noticeable at about the ninth week.

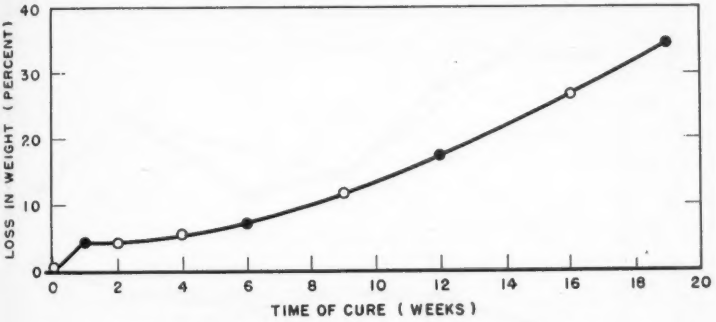

FIGURE 1.—Weight lost by sweetpotatoes during cure.

The weight-loss data covering the 19 weeks of cure are given in figure 1. After the first week, during which the rate of loss was greatest, there was a continuous and regular loss of weight until, at the end of the nineteenth week, the sweetpotatoes had lost over one-third of their weight.

The data in table 1 and the curves in figure 2 relate to changes in carbohydrate composition as the curing progressed. Curves *a*, *b*, *c*, and *d* follow changes in total carbohydrates, starch, nonreducing

TABLE 1.—*Weights of carbohydrates and solids in 100-gm.*[1] *samples of sweetpotato tissue*

Sample No.	Alcohol-insoluble solids	Alcohol-soluble solids	Total solids	Starch	Total sugars as invert sugar	Reducing sugars as invert sugar
	Grams	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
0	24.24	3.00	27.24	16.45	1.88	0.29
1	22.28	4.94	27.22	14.38	3.87	.54
2		5.22			4.05	.81
4	23.42	6.01	29.43	15.33	4.78	.49
6	21.50	5.82	27.32	13.80	4.65	.29
9	21.16	5.87	27.03	11.80	4.83	.33
12	23.49	5.90	29.39	12.45	4.70	.23
16	24.08	6.23	30.31	14.55	5.20	.23
19	25.96	7.88	33.84	14.40	6.72	.19

[1] Because of the variable amounts of water that the sweetpotatoes may have absorbed at the time of washing, the 100-gm. samples may not be strictly comparable in the sense that they reflect the water content of the sweetpotatoes at the end of the designated curing intervals.

sugars, and reducing sugars, respectively, in the total solids. Each carbohydrate fraction is defined only by the method of analysis and is reduced to a $C_6H_{10}O_5$ basis. The quantity designated as total carbohydrates is limited to starch plus total sugars calculated as starch. Total solids also are corrected for the sugar-bound water.

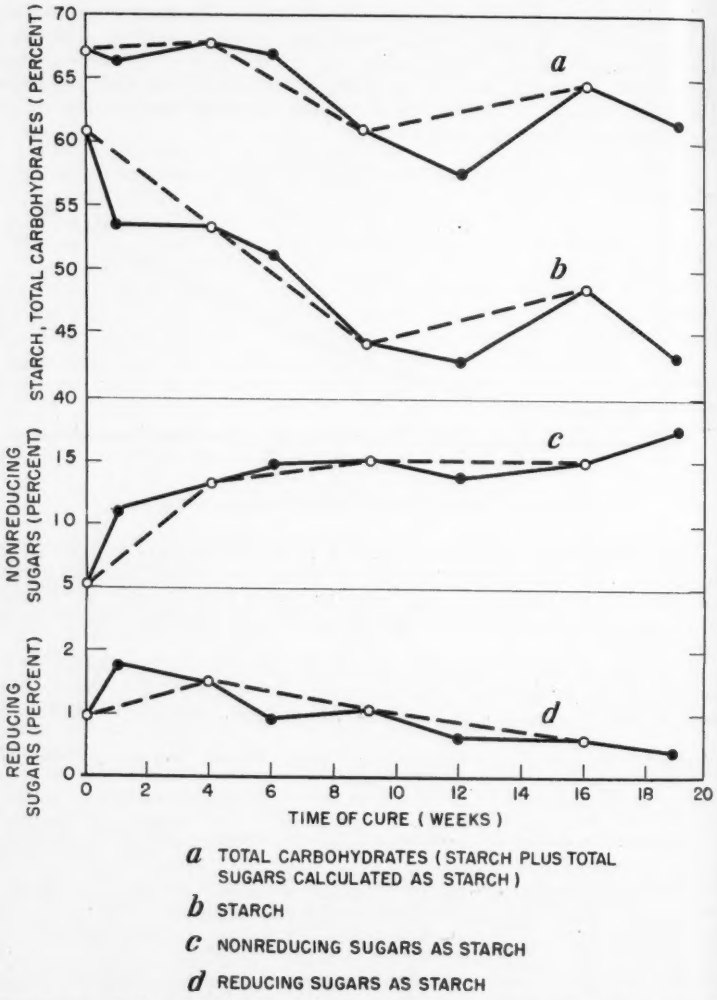

FIGURE 2.—Carbohydrate composition of sweetpotatoes during cure. Broken lines show effects that could reasonably be expected with no interruption of temperature and humidity control; solid lines, the effects of accidental lowering of temperature and humidity during cure, within the first-, sixth-, twelfth-, and nineteenth-week periods.

Under normal conditions of cure (broken line), the starch content decreased to the ninth week after which there was an increase to the sixteenth week. Nonreducing sugars increased continuously and regularly to the sixteenth week. Reducing sugars increased to the fourth week after which they decreased in a regular manner to the sixteenth week. No carbohydrate fraction responded uniformly to decreases in temperature and relative humidity (solid line). At the first week when the temperature first fell, there was a relative decrease in starch while both the nonreducing and reducing sugars increased. At the sixth week, the effect of lowering the temperature and relative humidity was to increase the starch and nonreducing sugars and to decrease the reducing sugars with respect to the amounts which would have been present in the tissue under normal conditions of cure. At the twelfth week, there was a relative decrease in all three

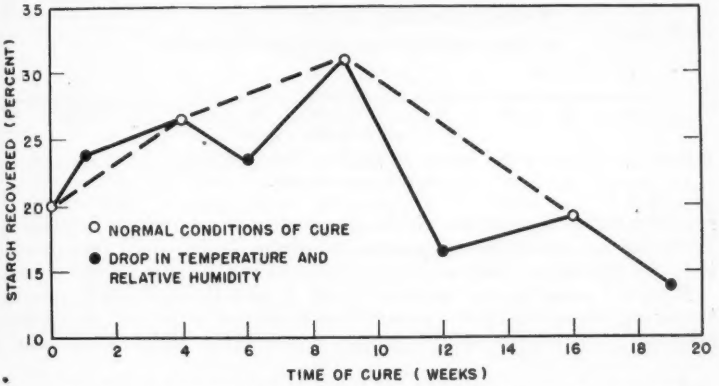

FIGURE 3.—Percent of total starch recovered at different times and under different conditions of cure.

carbohydrate fractions. Nonuniform responses to a lowering of the temperature can hardly be ascribed to the occurrence of enzymic hydrolytic changes in the tissue while it was being dried. The accumulation of sugars in sweetpotatoes while being heated at 66° C. (*11*) or cooked or dehydrated (after blanching) [4] is considerable unless enzymes are first inactivated. Total solids were determined only after treatment of the samples with 80-percent alcohol.

Under normal conditions of cure, the total carbohydrates increased slightly to the fourth week, decreased to the ninth week, and increased again to the sixteenth week. They also responded to a lowering of the temperature and relative humidity. There was a relative decrease in total carbohydrates at the first week, an increase at the sixth week, and a decrease at the twelfth week.

The curves shown in figure 3 express the percent of the total starch recovered as it was affected by the time and conditions of cure. The efficiency of recovery under constant conditions increased to the ninth week and decreased thereafter. The lowering of the

[4] Kansas Agricultural Experiment Station. Unpublished data.

temperature and relative humidity (solid line) increased the effectiveness of recovery during the first week but lowered it during the sixth and twelfth weeks. The combination of variable starch content and efficiency of recovery shown in figure 4 gives information which

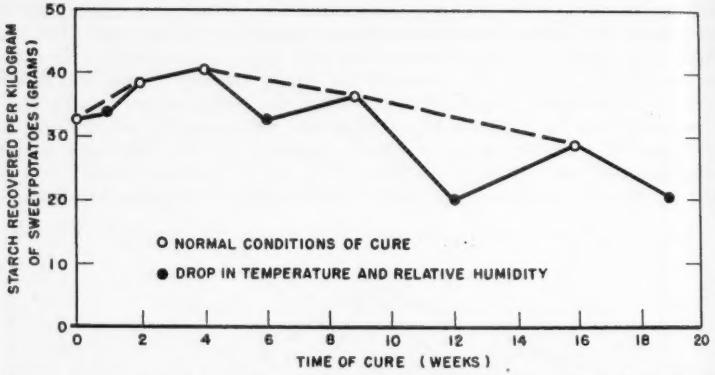

FIGURE 4.—Yields of dry starch obtained at different times and under different conditions of cure.

should have a bearing on yields of starch from cured sweetpotatoes With the curing methods commonly employed, it would be expected that the maximum yield would be obtained at the fourth week of cure.

Figure 5 presents the weights of the A and B fractions in relation to curing time. Under constant conditions of cure, the A fraction reached a maximum at the fourth week, after which there was a

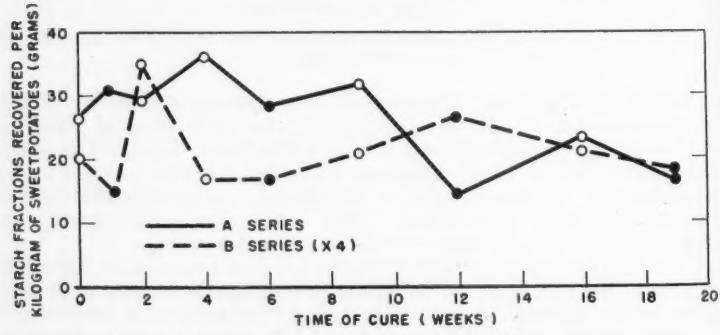

FIGURE 5.—Yields of dry starch fractions obtained at different times of cure.

steady decrease. The B fraction reached a maximum at the second week, dropped to its lowest value at the fourth week, increased appreciably to the ninth week, where it remained constant to the sixteenth week. Except at the sixth week, responses to a drop in temperature and relative humidity by the two fractions were in the opposite direction.

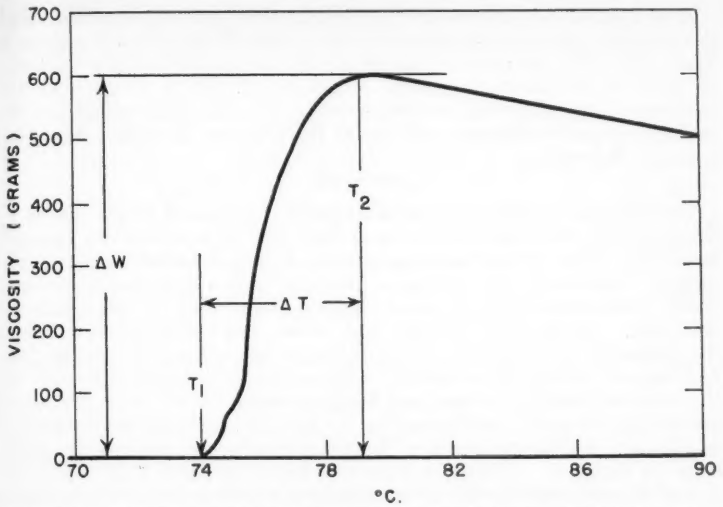

FIGURE 6.—Viscosity record of a sweetpotato starch during the heating stage: T_1, lower limit of gelatinization temperature; T_2, upper limit; ΔT, interval between these two temperatures; ΔW, initial maximum viscosity developed at T_2; and $\frac{\Delta W}{\Delta T}$, rate of gelatinization.

TABLE 2.—Temperatures at which viscosity in sweetpotato starch becomes measurable, T_1, and reaches its initial maximum, T_2; the intervals between these two temperatures, ΔT; the initial maximum viscosity developed at T_2, ΔW; and rate of gelatinization, $\frac{\Delta W}{\Delta T}$

Sample No.	Gelatinization temperature		ΔT ($T_2 - T_1$)	ΔW	$\frac{\Delta W}{\Delta T}$
	T_1	T_2			
	°C.	°C.	°C.	Grams	Grams per °C.
A-FRACTION STARCHES					
A-0	69.2	77.0	7.8	661	85
A-2	69.5	76.7	7.2	680	94
A-4	69.5	77.7	8.2	605	74
A-6	70.4	77.7	7.3	602	82
A-9	70.2	77.5	7.3	619	85
A-12	70.8	77.5	6.7	645	96
A-16	70.8	77.5	6.7	613	92
A-19	71.0	78.0	7.0	633	90
B-FRACTION STARCHES					
B-0	70.9	77.5	6.6	740	112
B-2	70.6	78.7	8.1	618	76
B-4	68.8	74.8	6.0	494	82
B-6	70.0	78.5	8.5	592	70
B-9	71.7	77.6	5.9	603	102
B-12	70.7	77.0	6.3	625	99
B-16	71.7	78.5	6.8	570	84
B-19	71.7	78.5	6.8	558	82

716802—46——2

A comparison of the starch content at different stages of cure with the average granule diameters of the A and B fractions is shown in figure 10. The pattern of change of the starch content was opposite to that of the average granule diameter of the A fraction to the sixth week and after the twelfth week; it was the same as that of the average granule diameter of the B fraction to the sixth week but opposite thereafter.

STARCHES

One means of following the changes in starches brought about by the time and variable conditions of cure was to measure the viscosity developed when 10-percent suspensions were gelatinized, cooked, and cooled. Although the complete pasting record was made for each starch, characterizations were based chiefly upon gelatinization behavior. In the text, graphs, and tables, the temperature at which the viscosity first becomes measurable is represented by T_1, the temperature at which the viscosity reaches its initial maximum by T_2, the interval between these two temperatures by ΔT, and the initial maximum viscosity developed at T_2 by ΔW. $\Delta W/\Delta T$ is designated as the rate of gelatinization. These quantities are presented graphically in figure 6. Viscosity data are found in table 2, in which the A and B series are identified by numbers which indicate the weeks of cure.

The solvent extraction of the granules was carried out only with the A series; at the time extractions were made, the quantities of the B series starches were not large enough to allow for their extraction and subsequent examination. The total material extracted from each starch sample was carefully collected, dried, and weighed. Further, protein analyses were run on each sample before and after solvent extraction. The nitrogen-protein conversion factor used was 6.25. The spread throughout the unextracted starches was between 0.06 and 0.11 percent as compared with 0.04 and 0.06 percent for the extracted starches. Figure 7 provides a comparison of the variation of

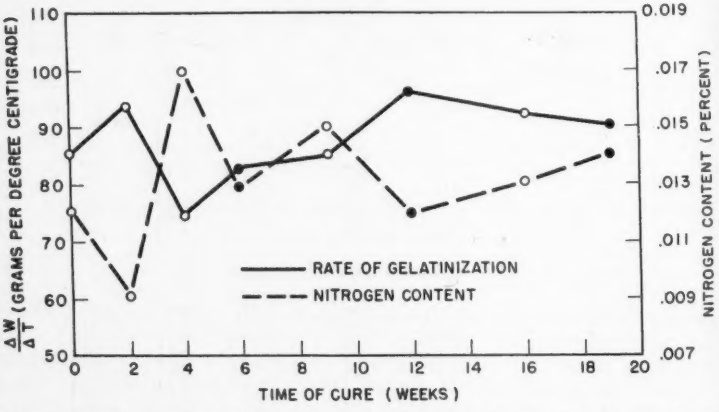

FIGURE 7.—Comparison of the changes in nitrogen content with changes in the rate of gelatinization of the A series of sweetpotato starches.

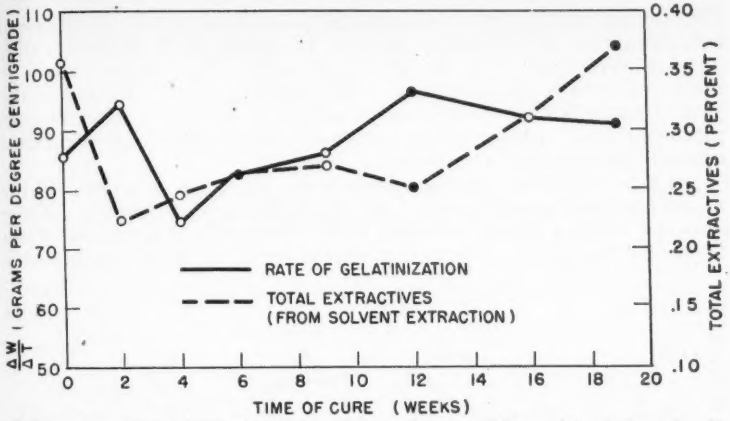

FIGURE 8.—Comparison of the changes in total extractives with changes in the rate of gelatinization of the A series of sweetpotato starches as influenced by the time and conditions of cure.

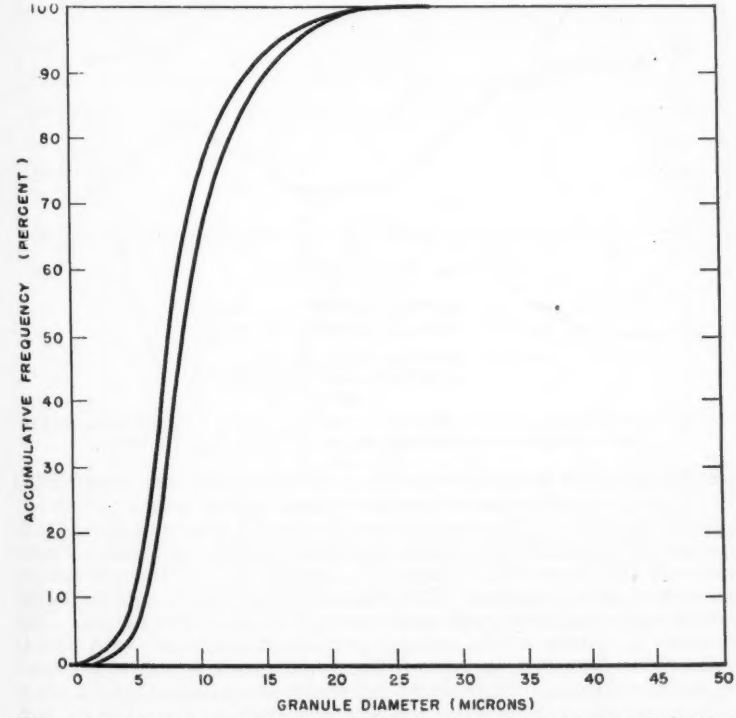

FIGURE 9.—Shifts in granule-size distribution of sweetpotato starch between successive curing intervals and before and after solvent extraction.

the nitrogen content of the unextracted starches with that of the rate of gelatinization ($\Delta W/\Delta T$); the patterns of change with respect to time of cure were inverse to the sixteenth week. The curves shown in figure 8 compare the changes in total extractives with changes in the rate of gelatinization of the starches as influenced by the time and conditions of cure. Except at the sixth week, these changes also exhibited opposite patterns up to the sixteenth week.

Granule diameter measurements were made on starches of the natural and solvent-extracted A series and the natural B series. These data are average values obtained from counts of 500 granules. Size-frequency histograms also were prepared for all of these starches from

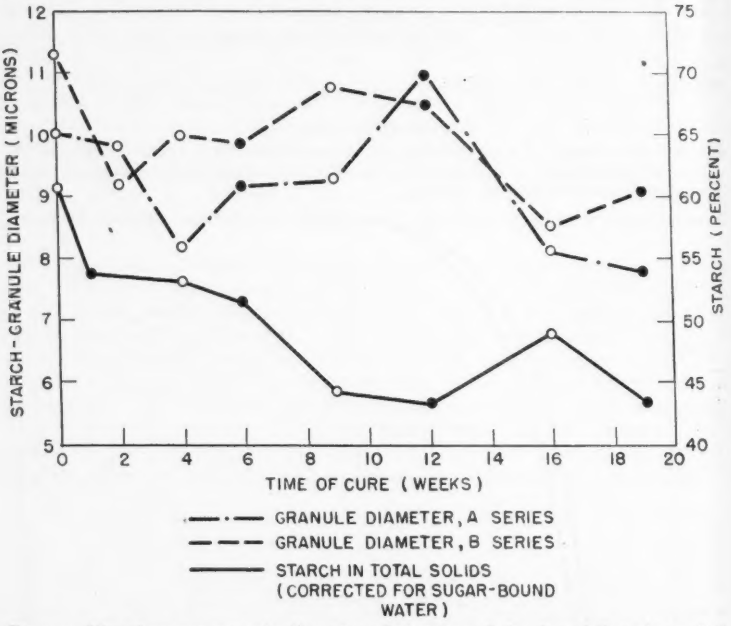

FIGURE 10.—Average granule diameter of starches of the A and B series and the starch content of sweetpotatoes, at different stages of cure.

granule diameter measurements, from which curves were constructed by plotting the midpoints of each size range against accumulative frequency (percent). The curves shown in figure 9 illustrate the shifts in granule-size distribution which occurred between successive curing intervals and those which came as a consequence of the solvent extraction of given starches. The granule-size displacements exhibited considerable regularity with respect to the various size ranges. The patterns of change of the average granule diameter in the A and B series of starches were opposite to the twelfth week but were the same thereafter (fig. 10). The solvent extraction of starches of the A series had the effect of increasing the average granule diameter in each case (fig. 12).

Figure 11, *A* and *B*, also provides a comparison of changes in average starch granule diameters of the A and B fractions, respectively, and rates of gelatinization, with time of cure. For both fractions, which showed opposite trends with respect to the granule diameter, the rate of gelatinization and the average granule diameter varied in much the

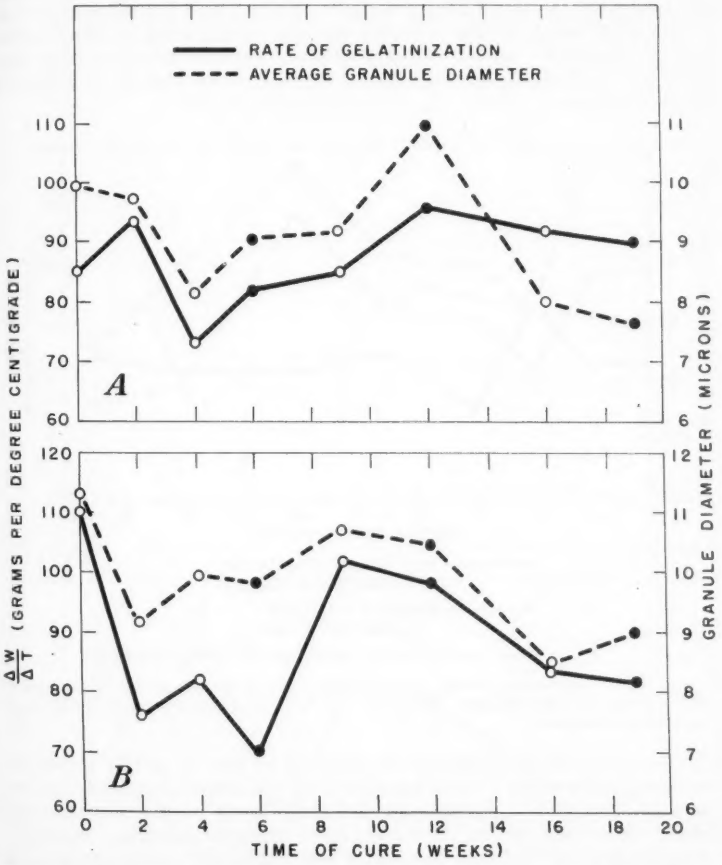

FIGURE 11.—Rate of gelatinization and changes in average granule diameters of sweetpotato starch at different stages of cure: *A*, A series; *B*, B series.

same manner. For the solvent-extracted A series, however, the patterns of change for the rate of gelatinization and the average granule diameter were opposite (fig.12).

Granule densities were measured in benzene and water. Densities measured in benzene were corrected for the water content of the starch before they were used in making comparisons. Densities measured in

water were used in calculating the water content of starch granules in contact with water, referred to later as the water-holding capacity. The calculation of the water-holding capacity was made on the assumption that the granule does not change in volume when it is placed in water. Gallay and Puddington (7) report no change in the volume of potato starch granules when held in water at 50° C. Granule densities (corrected to dry weight) of the A and B fractions, except at the sixth week in the A series and the ninth week in the B series (fig. 13, *A* and *B*), varied directly with the rates of gelatinization and, therefore, with the granule diameters (fig. 11, *A* and B) at different stages of cure.

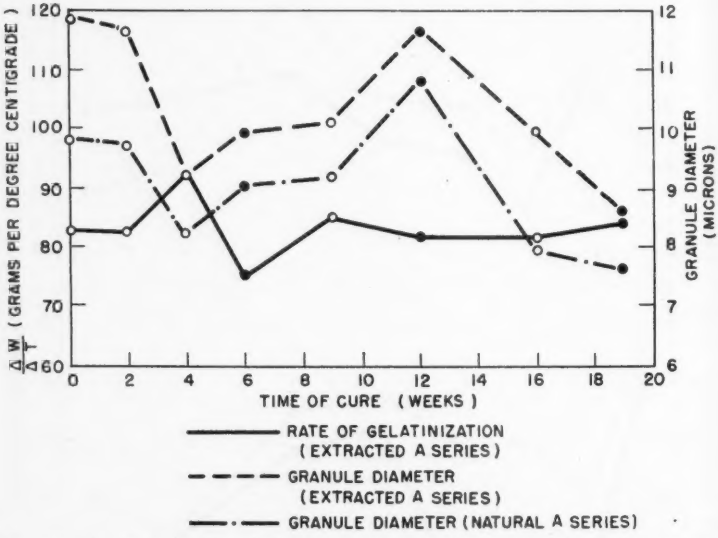

FIGURE 12.—Comparison of the granule diameters of the unextracted starches of the A series with the granule diameters and rates of gelatinization of the solvent-extracted starches.

The water-holding capacity of starches of the A and B series are compared with rates of gelatinization at different stages of cure in figure 14, *A* and *B*. Except for the sixth week in the A series and ninth week in the B series, the curves have opposite patterns. Further, it may be pointed out that the water content of the air-dried starches gave much the same pattern with respect to the rates of gelatinization. The spread between the water content of corresponding air-dried and water-suspended starches of both series was between 1.0 and 2.5 actual percent; the average difference was 1.47 actual percent. It is worthy of note that starches of both series varied in some predetermined manner in their water-holding capacity. This, together with the relatively small difference in the water content of air-dried and water-suspended starches, suggests that the water which entered was held within the granule as adsorbed water. In some respects, this is in

agreement with the view of Sair and Fetzer (*14, p. 206*) that "water is retained by starch between 20 and 90 percent R. H. in one form only—adsorbed water."

The curves of figure 15 show changes in the alkali lability with change in the rate of gelatinization ($\Delta W/\Delta T$) of the B series during cure. The patterns of change were inverse with respect to each other.

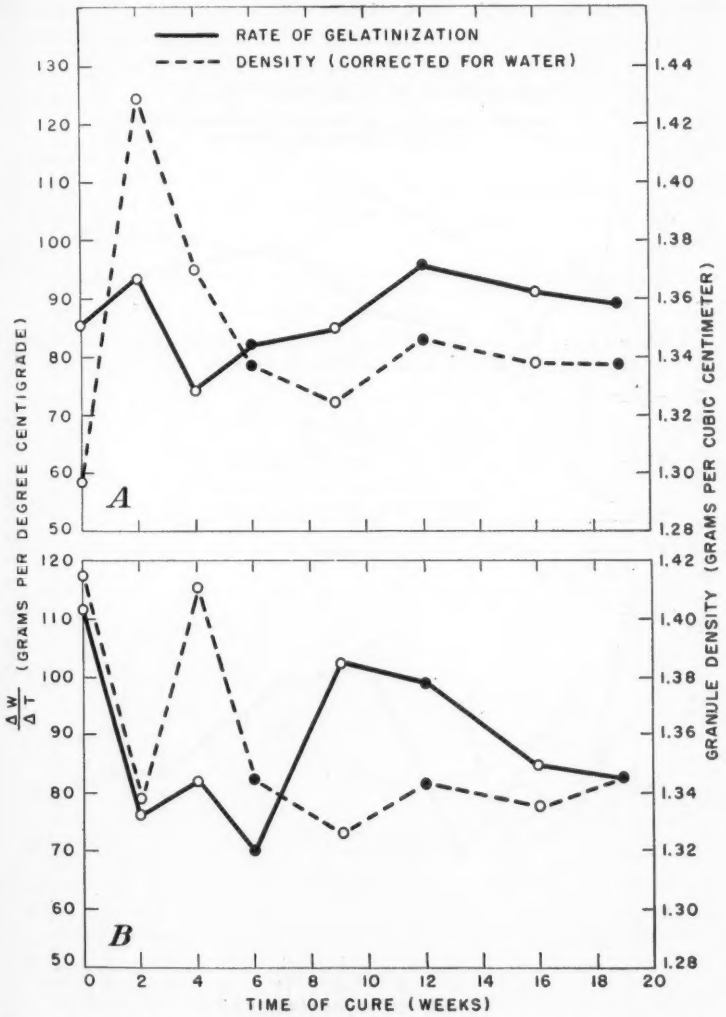

FIGURE 13.—Rate of gelatinization and corrected densities of sweetpotato starches at different stages of cure: *A*, A series; *B*, B series.

Tapioca starches obtained from different sources vary considerably, depending upon their history and purity. Figure 16 presents the viscosity records of three different tapioca starches and two sweetpotato starches. Curves *a*, *d*, and *e* show data for tapioca starches and curves *b* and *c* for sweetpotato starches. The tapioca starch of curve *d* is a natural starch of high quality and one which has undergone little change in storage; its viscosity record is typical of natural tapioca starch of good quality. The tapioca starch of curve *e* is a natural

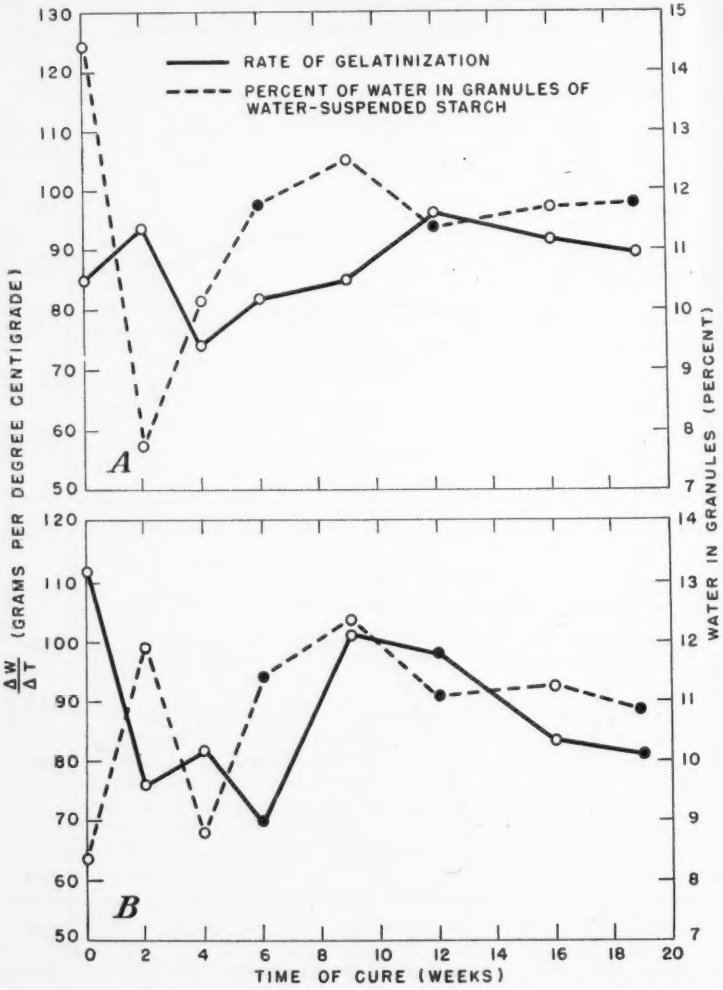
RATE OF GELATINIZATION
PERCENT OF WATER IN GRANULES OF WATER-SUSPENDED STARCH
A
B
$\frac{\Delta W}{\Delta T}$ (GRAMS PER DEGREE CENTIGRADE)
WATER IN GRANULES (PERCENT)
TIME OF CURE (WEEKS)

FIGURE 14.—Comparison of the rate of gelatinization and the water-holding capacity of sweetpotato starches at different stages of cure: *A*, A series; *B*, B series.

tapioca starch originally of high quality, but considerably changed through aging; its viscosity record 3 years ago was much the same as that of curve *d*. The tapioca starch shown in curve *a* has been chemi-

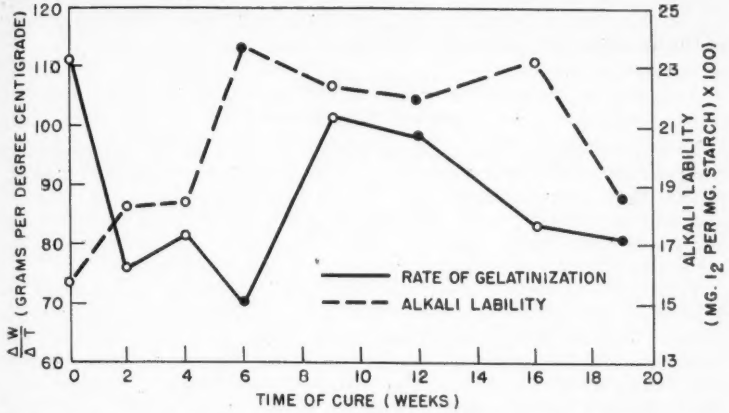

FIGURE 15.—Comparison of the rate of gelatinization and the alkali lability value of sweetpotato starches of the B series at different stages of cure.

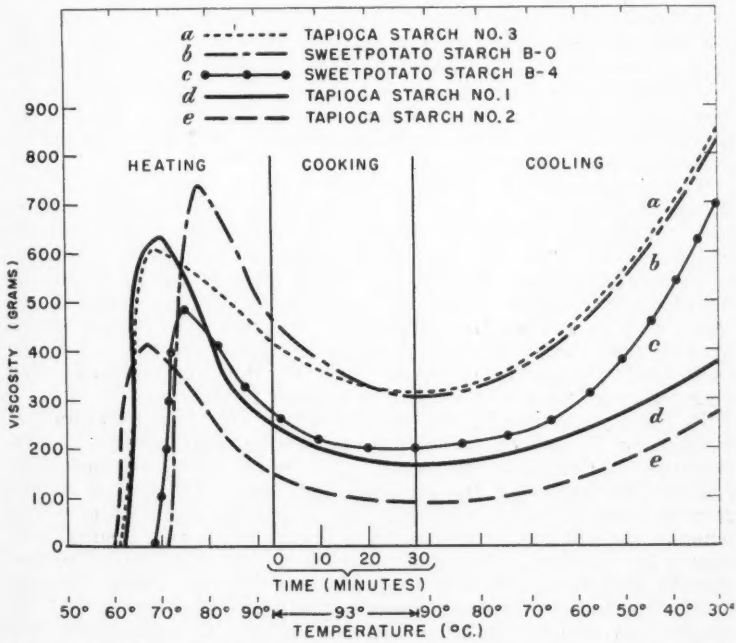

FIGURE 16.—Viscosity records of three tapioca and two sweetpotato starches.

716802—46——3

cally modified to convert it into a starch better suited for food uses. All three are commercial starches. The sweetpotato starch of curve *b* is the B fraction obtained from the uncured sweetpotatoes (B–0); that of curve *c* is the B fraction obtained at the fourth week of cure (B–4). These two were selected because they represent the extremes in quality of the sweetpotato starches obtained.

DISCUSSION

SWEETPOTATO TISSUE

In a discussion of the changes which occured in the carbohydrate composition of the sweetpotatoes as a result of the conditions imposed, it should be emphasized that the carbohydrate fractions—starch, nonreducing sugars, and reducing sugars—are defined only by the analytical procedures used for their estimation. No attempts were made to identify individual components of these fractions.

Very little is known about the number and nature of enzymic reactions involving carbohydrates that take place in situ in sweetpotato tissue. Polysaccharide-sugar interconversions and respiration are the most generally recognized. In the case of the former, polysaccharides other than starch have been given little consideration. Furthermore, there appears to be no direct evidence with respect to the identity of the sugars that are in equilibrium with and that represent the end products of the hydrolysis of the polysaccharides. Barker (*5*), in a study of potatoes, established that, within the temperature limits of 1° to 10° C. and except at high sucrose concentrations, curves relating the rate of respiration with the concentration of extractable sucrose closely resemble those which characterize enzymic reactions in vitro. His conclusion was that, in potatoes, sucrose either is or determines the respirable substrate. It is to be presumed that one or more of the polysaccharides constitute the ultimate source of the sucrose but its immediate origin is not known.

The component of the tissue that is most readily measured is water, although the accuracy of its estimation may be open to question. Aside from chemical equilibria involving water, the processes which directly affect the over-all tissue-water content are transpiration, imbibition, and respiration. Since little is known about the distribution of water within the structurally distinct parts of the tissue and its availability for reaction, the effects of change in the over-all tissue-water concentration upon a given reaction cannot be predicted. It may be expected, however, that the effects of such changes would vary with structural changes in the tissue.

In these experiments, the rate at which the sweetpotatoes lost weight (fig. 1) showed no response to simultaneous changes in temperature and relative humidity. All other measured quantities reflected variations in the conditions of cure. The loss in weight may be regarded as a composite loss of carbon dioxide and water, whereas each of the other quantities is concerned with a single substance (or fraction). Not only does the weight loss involve carbon dioxide and water but also it is possible that the water, through imbibition and transpiration, may make such adjustments that the weight loss rate is regular in spite of changes in temperature and relative humidity.

The curves of figure 2 may be considered as evidence of the complexity of participating carbohydrate reactions and of the factors that affect their rates. It is generally believed that a drop in temperature occasions a decrease in starch and an accumulation of sugars. This occurred at the first week when the temperature first decreased but not at the sixth and twelfth weeks. Either of the sugar fractions might have contained the respirable substrate, depending upon the relative rates of the carbohydrate interconversions and respiration. The most notable observation to be made with reference to the changes in carbohydrate composition is that the total carbohydrates (starch plus total sugars calculated as starch) increased slightly to the fourth week and markedly from the ninth to the twelfth week. This behavior requires the existence in the tissue of some carbohydrate which either is not hydrolyzed at all or is incompletely hydrolyzed during analysis but which is convertible in situ to carbohydrates that, in some degree, are measurable by analysis. The reversibility of the reaction is suggested by the simultaneous decrease of all three carbohydrate fractions when the temperature dropped at the twelfth week. That some starch ultimately came from this source is indicated by the increase in starch under constant conditions of cure between the ninth and sixteenth weeks while the nonreducing sugars remained constant and the reducing sugars decreased.

Not only are there indications of a difficultly hydrolyzable carbohydrate that participates in the carbohydrate balance in the tissue but there is also evidence that the carbohydrate substance from one part of the tissue may be transferred to another, possibly from one morphological environment to another.

At the outset the purpose of the preparation and the separate examination of starches of the A and B series was to increase the yield of starch and to learn whether or not the corresponding fractions were identical. If they were not identical, it would mean that a fractionation of the starch had taken place, presumably because of the failure to carry out the simple procedure used in preparing it in a reproducible manner. To those experienced in such procedures, there would be an inclination to dismiss any dissimilarities within the fractions as purely accidental. However, in view of the patterns of change that the series follow during cure and their relationships with each other and the starch content, it is possible that the two series of grinds made in this study yielded, without excessive overlapping, two starch fractions having their origin in morphologically distinct parts of the tissue.

The patterns of change of the corresponding A and B fractions were opposite with respect to a number of properties. This is true of their actual weights (fig. 5). To say that as one fraction increases, the other decreases, is not a statement of the obvious; either fraction could have been increased from the relatively large amount of residual starch in the tissue or from some reserve carbohydrate.

An inspection of figure 10 shows that the A and B series of starches exhibited opposite trends in the variation of the average granule diameter during the first 12 weeks of cure. That they were the same subsequent to the twelfth week might be expected in view of the evidence of structural changes in the tissue during the later stages of

cure. It will be noted also that the variation in starch content was not consistently comparable with that of the average granule diameter of either the A or B series of starches.

One interpretation of the opposite patterns of the average granule diameters of the two series is that the fractions may be identified with separate and distinct parts of the tissue and that one may have been formed at the expense of the other. There is the suggestion also that, of the two fractions, the B fraction made the greater contribution to changes in starch content during the first six weeks and the A fraction from the sixth to twelfth weeks. Further, there is an indication that competing starch-sugar interconversions were contributing to the nonuniformity of response of the starch content to a lowering of the temperature; at the sixth week, there was a relative increase rather than a decrease in the starch content as a result of a drop in temperature.

In accounting for the relationships between the average granule diameters of the two series of starches, it might be argued that they could be due to nonuniform changes in the granule-size distribution. Figure 9 shows this to be improbable. The granule-size distribution curves typify the shifts which occurred at succeeding intervals of cure. Shifts from one distribution to another were regular with respect to the complete range of diameters and indicate that enzymic changes involving the granules were essentially statistical rather than specific. It therefore seems probable that starch was hydrolyzed and laid down continuously in the tissue and that a change in conditions altered the rates of the various processes with respect to each other.

Other granule properties which varied in a similar manner with time of cure and which, therefore, distinguish between the A and B series of starches, are the rate of gelatinization (fig. 11, *A* and *B*), the density (fig. 13, *A* and *B*), and the water-holding capacity—percent water in the granules in contact with water (fig. 14, *A* and *B*). The last two may be dependent variables although not essentially so. Thus, there is definite reason to regard the two series as separate and distinct and not as having resulted from fortuitous fractionations which bear no relation to cellular structure.

Küntzel and Doehner (*10*) have reported that there are two phases in the development of pastes during the heating of starch suspensions. The first phase consists of the formation of a melt from starch crystallites and the deformation of starch molecules. The second phase involves the hydration (swelling) of the molecules thus liberated. The first is an endothermic, the second an exothermic process. This concept comes as close as any other to representing commonly held views of paste formation. In particular, it carries the implication that swelling occurs throughout the granule rather than at the surface and that the high viscosities developed during paste formation come from interference between swollen granules. However, it contributes little information relative to the influence on the gelatinization process and subsequent paste behavior of granule diameter, percentage of starch in the granule, water-holding capacity of the granule, density, substances adsorbed within the granule, and alkali lability.

The effect of granule diameter on the viscosity of a paste has been variously reported in the literature. Some investigators have claimed

that the viscosity is directly related to the granule diameter; others have held that the relation is indirect. A direct relation between the viscosity and granule diameter would imply that the accessibility of water to those areas at which gelatinization occurs is such that the extent of swelling approaches a maximum. An indirect relation, on the other hand, would suggest that the extent of swelling is so restricted that it appears to be dependent upon the peripheral surface area of the granule.

In these experiments it was found that the rates of gelatinization measured under given conditions and granule diameters of the natural starches (fig. 11, *A* and *B*) followed the same trends during the curing period. For this reason it is concluded that, in these instances, the rate of gelatinization was directly proportional to the granule diameter and that, therefore, the ease at which water is made available for swelling is not greatly altered as a consequence of the tissue changes which occur throughout the curing period. Evidence of this direct relation becomes still more convincing when it is recalled that it applies to two fractions of an opposite character.

In a consideration of the rate at which starch will gelatinize under given conditions, it would seem logical to assume that this rate would increase as the weight of starchy substance per unit volume increased. With starches whose granule contents are equally available for swelling, the rate of gelatinization should be greater the greater the density. The data presented in figure 13, *A* and *B*, show that the density of starches of the A and B series, corrected to dry weight, varied directly with the rate of gelatinization at different stages of cure.

It is equally reasonable to assume that the rate of gelatinization would be inversely proportional to the water-holding capacity or the water content of the granules in contact with water. At first glance, this might seem to be a corollary to the relation between density and rate of gelatinization mentioned above. However, the function of water in the swelling process is doubtless complex and in some respects must vary independently of the density. It is possible that an increase in firmly bound water may increase or decrease the rate of gelatinization, depending (1) on the extent to which the water, as a measure of crystallite formation, predetermines the amount of swelling or (2) on the extent to which the endothermic heat requirements lessen the reaction rate. An increase in loosely bound water also may increase or decrease the gelatinization rate, depending on the extent that the water is used in hydration or the extent to which it is a measure of that fraction of the granule which does not directly participate in the swelling process. No attempts were made to distinguish between the different types of adsorbed water but the experimental results suggest that in this case the latter function of the loosely bound water is the more significant. Figure 14, *A* and *B*, shows that the water-holding capacity varied indirectly with the rate of gelatinization.

Starches of commerce contain various amounts of noncarbohydrate organic substances, depending upon their origin and method of manufacture. The greater part of these substances are adsorbed and may be removed by solvent extraction. In considering their possible effects on the viscosity, the inclination would be to assume that

they would modify the rate and possibly the extent to which water is available for swelling. Rundle and Edwards (*13*) have reported that the helices of starch molecules apparently tighten with the adsorption of iodine and of butanol. The adsorption of other molecules of the proper dimensions might do likewise. If such is the case, starch granules would be expected to decrease in diameter with increasing amounts of adsorbed substances. Further, the consequences of adsorption would be expected to modify the rate at which water is available for gelatinization.

An examination of figures 7 and 8 shows that both the protein and total extractives decreased with increasing rate of gelatinization at different stages of cure. It is possible that the increasing rate of gelatinization is due entirely to the increase in granule diameter (fig. 11, *A*) brought about by mechanical effects, but it is not probable. Structural changes in the granule should also be taken into account.

The prevailing opinion regarding the significance of alkali lability is that it is a measure of reducing groups. Its use has been as an index of hydrolysis. Since the Taylor method for measuring alkali lability depends on a reaction of the carbohydrate substance with iodine and the Schoch method depends upon a reaction with sodium hydroxide, these methods will be in error to the extent that iodine or base-consuming extraneous substances are present in the starch. The amounts of noncarbohydrate substances vary to a considerable extent in natural starches but are greatly reduced by solvent extraction. In these experiments, the trend of the alkali lability during cure was opposite to that of the rate of gelatinization (fig. 15) and therefore followed the trend of the protein and total extractives (figs. 7 and 8).

In the case of natural starches, the trends of change during cure indicate not only that the granule diameter increased with a decrease in the amount of adsorbed organic substances but also that solvent extraction actually caused an increase in the granule diameter (figs. 9 and 12). When the solvent-extraction method was used, however, there was a further increase in alkali lability,[5] which could no longer be ascribed to normally present extraneous adsorbed substances, and the rate of gelatinization varied indirectly instead of directly with the granule diameter (fig. 12). The latter fact suggests that increased alkali lability is associated with a reduced rate of availability of water for swelling. Support for this statement is provided by data obtained by Schrenk,[6] who found that, whereas solvent extraction has no effect on the magnitude of the heat of wetting of a starch, it markedly decreases the rate at which wetting occurs. The results set forth above indicate that solvent extraction causes a structural change in the granule. It should be added, however, that the results of increased alkali lability with solvent extraction as here reported are not in agreement with those of Schoch (*15*), who found the opposite to be true in the case of cornstarch. The possibility that traces

[5] The comparison of the alkali lability of the solvent-extracted A series starches was not followed beyond the sixth week of cure since samples of this series had been exhausted.

[6] SCHRENK, W. G. A CALORIMETRIC STUDY OF CERTAIN STARCHES, GLUTENS, AND FLOURS IN WATER AND WATER SOLUTIONS. 1945. [Unpublished doctor's thesis. Copy on file, library, Kansas State College, Manhattan.]

of adsorbed methanol remain in the solvent-extracted starches can scarcely account for the increased alkali lability by the Taylor method; similar results were obtained by the Schoch method.

In the foregoing paragraph, evidence was presented showing that, for natural sweetpotato starches which allow water to become available for gelatinization at a rate in excess of some minimum value, the rate of gelatinization is governed directly by the granule diameter and the density and indirectly by the water-holding capacity, the quantities of adsorbed organic substances, and alkali lability. Furthermore, substances adsorbed within the granule influence the rate of gelatinization by exercising some kind of control over the rate at which water becomes available for swelling; in other words, the lower the content of adsorbed organic substances the more readily the water becomes available. The enzymic modification of the quality of starch in sweetpotato tissue during cure seems to be governed principally by these factors. All starches respond to fluctuation in temperature and relative humidity. With a given change in curing conditions, the quality of one part of the recoverable starch will become altered in one direction and that of another in an opposite direction. Should there be any occasion for it, the quality of the bulk of the starch can be predetermined within limits by controlling the curing or storage conditions. Insofar as is known, however, such a procedure would be limited to the factors mentioned. Judging by the regularity of patterns of change there appears to be little change in the basic granule structure of starch, which is concerned with the variability of molecules and their association with each other.

COMPARISON OF TAPIOCA AND SWEETPOTATO STARCHES

It is not yet possible to compare starches of different origin on a strictly quantitative basis. Differences between them include not only variations in granule diameter, density, and water-holding capacity but variations in basic structure as well. The constituent molecules of starch granules are usually classed as branched and linear. These two types of molecules may vary from starch to starch in relative amounts, size and shape, and the manner of their association in the granule. It is probable that the last named is a factor in determining the extent and type of crystallite formation.

Of the many methods that have been used for studying starch, viscometric methods have been employed most extensively. In the past, the usual procedure has been to make single-point measurements of viscosity and, as a consequence, errors have arisen from comparing pastes at different stages of development. Continuous measurements of viscosity during and subsequent to the development of the paste make possible a study of the factors that contribute to paste viscosity. Some interpretation of the pasting record is already possible but it is far from complete. A continued study of these contributing factors will be required before an adequate evaluation of a starch can be made by viscosity alone.

One of the characteristics of a starch of a given origin which is difficult to change is the lower limit of the gelatinization temperature. This is illustrated in figure 16 which shows that the tapioca starches of different history begin gelatinizing at about 60° C. and sweetpotato

starches at about 70°. It is true that curing has had the effect of shifting gelatinization toward lower temperatures to the extent of more than 2°, but this is not sufficient to justify the claim that sweetpotato starch has acquired a distinct increase in tapioca like character. It is believed that the persistence of this quality in starches of a given origin is due to the basic granule structure.

However, when it is a question of the peak viscosity developed during pasting, sweetpotato starch can be modified during cure so that it more closely resembles tapioca starch. Doubtless, this can be done with other starches, since modification probably results from changes in granule diameter, density, water-holding capacity, and permeability without any considerable change in basic structure. Tapioca starch No. 1, data for which are presented in figure 16, is a typical natural tapioca starch of good quality. Starch No. 2 was of equal quality 3 years ago. During that time, aging caused about the same drop in the initial maximum viscosity of tapioca starch as curing did for sweetpotato starch. One of the factors which contributed to this, in the case of sweetpotato starch, was the granule diameter; possibly also this accounts in part for differences in the pasting behavior of the two natural tapioca starches. In any case the average granule diameter of tapioca starch No. 1 is 11.20μ while that of the aged tapioca starch is 10.55μ; its granule diameter was not measured in its original condition.

A predominant characteristic of natural tapioca starch is its "gumminess." Qualitatively, this character is more pronounced the higher the initial maximum viscosity and the lower the viscosity of the developed paste at room temperature. The five starches for which data are given in figure 16 rank, in the order of decreasing gumminess, as follows: Tapioca starch No. 1, tapioca starch No. 2, sweetpotato starch B–0, tapioca starch No. 3, and sweetpotato starch B–4. "Cook-up" tests, which are commonly used for evaluating starches, gave them the same rank.

Tapioca starch No. 3 is one which has been chemically modified to improve its quality for food uses. It has been made "shorter," but not to the extent that it will not form a smooth paste upon stirring. The change in shortness has made it intermediate between the sweetpotato starches, with a greater similarity to B–4 than to B–0.

In summarizing it may be said that the quality of sweetpotato starch is modified in the root as it undergoes cure. This modification seems to be the result of changes in granular diameter, density, water-holding capacity, and permeability and not to changes in the basic structure of the granule. Sweetpotato starches in general resemble tapioca starches and there is no evidence to indicate that the curing of sweetpotatoes substantially enhances this similarity.

SUMMARY

Sweetpotatoes, cured for a period of 19 weeks, lost about 34 percent of their weight. During that time they became brown in color, woody in texture, wrinkled, and acquired a density of less than 1. They were free from rot.

Decreases in temperature and relative humidity which occurred during the curing period were accompanied by fluctuations in the

reducing sugars, nonreducing sugars, and starch. Changes in the carbohydrate composition suggest that some carbohydrate substance not completely measured by the direct acid hydrolysis method was present and participated in the carbohydrate balance in the tissue.

Two starch fractions (A and B) were prepared at different stages of cure and these, because of their behavior, were judged to be of different origin. The fraction of the total starch recovered at any stage did not exceed 31 percent. The patterns of change of the two fractions were opposite with respect to their actual weights, granule diameter, density, water-holding capacity, and rate of gelatinization.

At times the starch content of the tissue varied in a manner similar to that of the average granule diameter of the A series of starches, and at other times similar to that of the B series. Furthermore, when there was an increase or a decrease in the average granule diameter of either starch fraction between successive curing intervals there was a shift in the complete range of granule diameters. These results indicate that there was a transfer of carbohydrates from one part of the tissue to another during cure.

Judging by the patterns of change during cure, modification of starch quality was brought about by changes in granule diameter, density, water-holding capacity, and substances adsorbed within the granule. If the basic structure of the granule was altered, the alteration was not significant. It was found that the rate of gelatinization was governed directly by the granule diameter and the density and indirectly by the water-holding capacity, the amounts of adsorbed organic substances, and alkali lability. The granule diameter increased as the content of adsorbed substances decreased.

There was no evidence to indicate that the curing of sweetpotatoes enhanced the tapiocalike quality of the starch through an alteration of the basic granule structure.

LITERATURE CITED

(1) Association of Official Agricultural Chemists.
1940. official and tentative methods of analysis . . . Ed. 5, 757 pp., illus. Washington, D. C.

(2) Barham, H. N., Kramer, G., and Reed, G. N
1943. influence of various factors on the starch content of kansas-grown potatoes and sweetpotatoes. Jour. Agr. Res. 67: 395–406, illus.

(3) ——— Wagoner, J. A., and Reed, G. N.
1942. viscosity of starches. determination in a rotating cylinder viscometer. Indus. and Engin. Chem. 34: 1490–1495, illus.

(4) ——— Wagoner, J. A., Williams, B. M., and Reed, G. N.
1944. a comparison of the viscosity and certain microscopical properties of some kansas starches. Jour. Agr. Res. 68: 331–345, illus.

(5) Barker, J.
1936. analytical studies in plant respiration. vi. the relation of the respiration of potatoes to the concentration of sugars and to the accumulation of a depressant at low temperatures. part 3. the relation of the respiration to the concentration of sucrose. Roy. Soc. London Proc., Ser. B., 119: 453–473, illus.

(6) Etheredge, M. P.
1941. a survey of methods for the quantitative estimation of starch. Assoc. Off. Agr. Chem. Jour. 24: 113–119.

(7) Gallay, W., and Puddington, I. E.
1943. the hydration of starch below the gelatinization temperature. Canad. Jour. Res., Sect. B, 21: 179–185, illus.
(8) Gooden, E. L., and Smith, C., M.
1940. measuring average particle diameter of powders. an air-permeation apparatus Indus. and Engin. Chem., Analyt. Ed. 12: 479–482, illus.
(9) Jacobs, M. B.
1938. the chemical analysis of foods and food products. 537 pp., illus. New York.
(10) Küntzel, A., and Doehner, K.
1939. untersuchungen über die verkleisterung der stärke. 1. lichtelektrische und ultramikroskopische analyse der verkleisterung. Kolloid Ztschr. 86: 124–130, illus.
(11) Niflsen, J. P., and Gleason, P. C.
1945. rapid determination of starch. factors for starches and comparison with acid and enzymic hydrolysis methods. Indus. and Engin. Chem., Analyt. Ed., 17: 131–134, illus.
(12) Paine, H. S., Thurber, F. H., Balch, R. T., and Richee, W. R.
1938. manufacture of sweetpotato starch in the united states. Indus. and Engin. Chem. 30: 1331–1348, illus.
(13) Rundle, R. E., and Edwards, F. C.
1943. the configuration of starch in the starch-iodine complex. iv. an x-ray diffraction investigation of butanol-precipitated amylose. Amer. Chem. Soc. Jour. 65: 2200–2203, illus.
(14) Saip, L., and Fetzer, W. R.
1944. water sorption by starches. Indus. and Engin. Chem. 36: 205–208, illus.
(15) Schoch, T. J.
1942. non-carbohydrate substances in the cereal starches. Amer. Chem. Soc. Jour. 64: 2954–2956.
(16) ——— and Jensen, C. C.
1940. a simplified alkali-lability determination for starch products. Indus. and Engin. Chem., Analyt. Ed. 12: 531–532, illus.
(17) Taylor, T. C., Fletcher, H. H., and Adams, M. H.
1935. determination of the alkali-labile value of starches and starch products. Indus. and Engin. Chem., Analyt. Ed. 7: 321–324, illus.

BACTERIAL SPOT OF NATIVE GOLDEN CURRANT (RIBES AUREUM)[1]

By G. W. Bohn, *associate pathologist*, and J. C. Maloit, formerly *agent*, *Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture* [2]

INTRODUCTION

Since 1939 a bacterial spot has caused severe defoliation of native golden currant (*Ribes aureum* Pursh) in experimental plantings at the United States Department of Agriculture Cheyenne Horticultural Field Station, Cheyenne, Wyo. In 1940 and 1941 the disease was severe enough to cause the loss of all leaves except those produced on new shoots late in summer. This disease has been observed in ornamental plantings in Cheyenne and in plants which occur naturally in the Pole Mountains west of Cheyenne. It causes plants to be unsightly in ornamental and windbreak plantings and reduces yields directly by fruit infections and probably indirectly by weakening the plants through the loss of leaves.

A review of the literature on diseases of currants revealed several leaf spots (*6*, *11*, *12*, *14*, *17*),[3] [4] but none that was reported to be caused by bacterial pathogens. So far as the writers know the only reports of parasitic bacteria on *Ribes* are those concerned with crown gall (*3*, *p. 22; 10*, *p. 38; 13*, *p. 586; 20*) and a blossom blight (*16*), both on gooseberry. No other information has been found to add to the statement "two bacterial diseases have been reported on Ribes" made in 1920 by Smith (*18*, *footnote p. 7*). It is probable that Smith referred to crown gall and blossom blight of gooseberry. Since no description of the bacterial spot of golden currant has appeared in the literature, it and its causal organism are described in this paper.

[1] Received for publication May 14, 1945.

[2] The writers wish to thank H. A. Edson, formerly principal pathologist in charge, Division of Mycology and Disease Survey, Bureau of Plant Industry, Soils, and Agricultural Engineering, for assistance in obtaining references on bacterial diseases of *Ribes*, and W. H. Burkholder, professor, Department of Plant Pathology, Cornell University, Ithaca, N. Y., for helpful criticism of the technical description of the pathogen and for the data on its effects on lipoids, sodium tartrate, and tyrosine. Thanks are extended also to the following members of the staff of the United States Department of Agriculture Cheyenne Horticultural Field Station for the services indicated: A. C. Hildreth, formerly principal plant physiologist and superintendent, and A. W. Krofchek, formerly agent, for supplying plants of *Ribes;* Gerald Brown, junior chemist, for determinations of the H-ion concentrations of carbohydrate and other media; and J. V. Hastings, formerly clerk, for making the photographs.

[3] Italic numbers in parentheses refer to Literature Cited, p. 289.

[4] Weiss, F. Check list revision. U. S. Bur. Plant Indus., Plant Dis. Rptr. 26: 176–189. 1942. [Processed.] (See pp. 179–185.)

THE DISEASE

Bacterial leaf spots on native golden currant are usually 2 to 4 mm. in diameter, but isolated spots are frequently larger. Spots in the blade are circular; those near large veins are irregular in shape. Well-developed spots have dark-brown, slightly depressed centers and reddish-brown, water-soaked, slightly raised margins. The raised margins are surrounded by narrow halos with distinct, entire or minutely undulate edges or by broad halos with indistinct edges (fig. 1, *A*). Under some conditions the margins grade into slightly

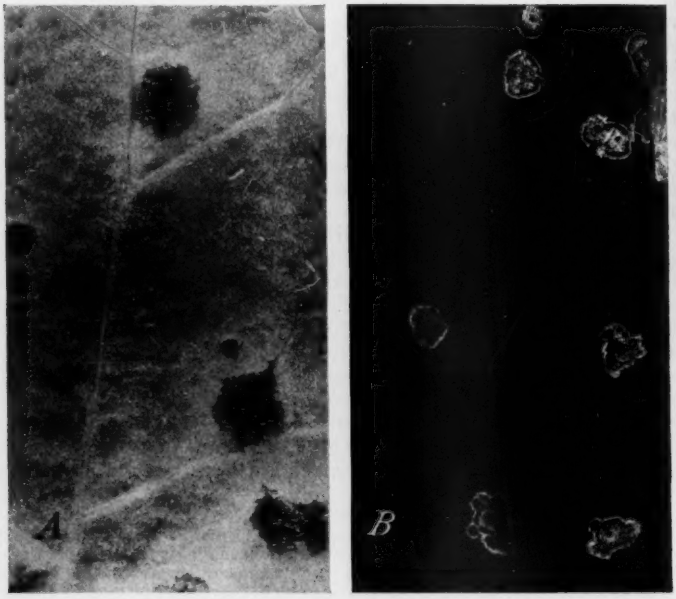

FIGURE 1.—Leaf spots on *Ribes aureum* resulting from brush inoculation with *Pseudomonas ribicola*, photographed on panchromatic film: *A*, In reflected tungsten light with a green Wratten (No. 58) filter; *B*, in transmitted tungsten light without a filter. *A*, × 6; *B*, × 3.

raised, dark-green, water-soaked areas with irregular, indistinct margins. Small veins within spots are discolored reddish brown and shriveled. This discoloration may extend short distances beyond the spot margins in the blade. Spots involving veins sometimes coalesce; those in the blade rarely do.

The spot shows a definite pattern by transmitted light (fig. 1, *B*). Bacterial masses at the inoculation point in the center and at the inner edge of the raised margin stop light. The remainder of the depressed center is moderately translucent, and the remainder of the raised margin is more translucent. If a piece of a leaf containing a spot is placed on a glass slide, cut through the center of the spot, covered with water and a cover slip, and examined with a microscope, masses of

bacteria can be seen to exude from the cut edges of the central point and the raised margin.

Infection occurring early in the ontogeny of the leaf results in distortion and tearing. Leaves with several spots turn various shades of yellow and red with green zones remaining around the spots (fig. 2, *A*). Severely spotted leaves soon turn brown, dry, and fall. Leaves

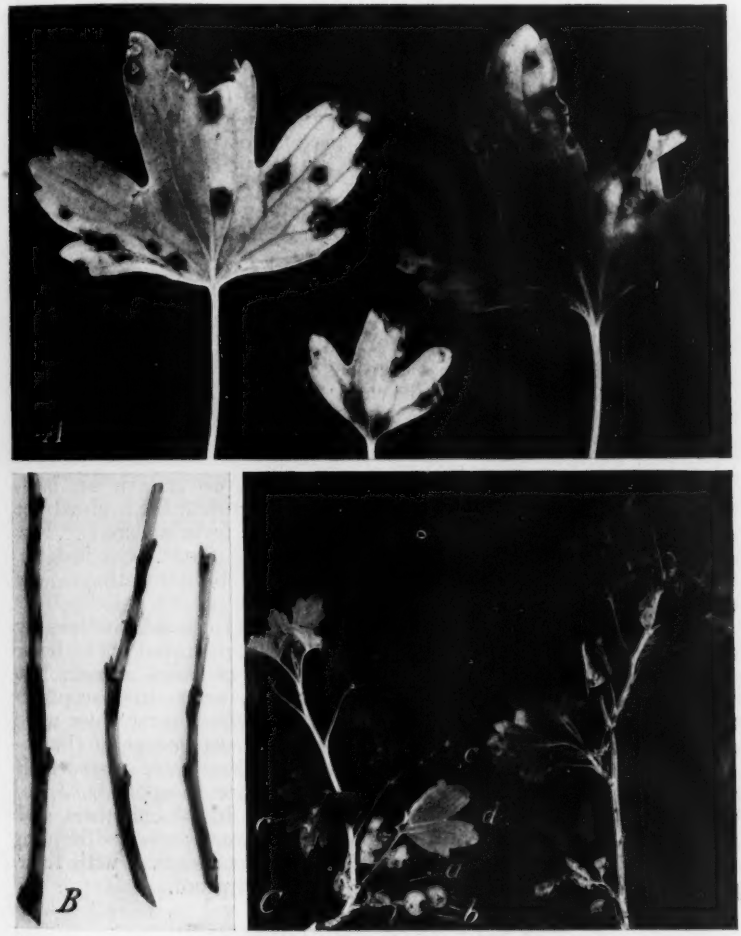

FIGURE 2.—*Ribes aureum* naturally infected by *Pseudomonas ribicola*, photographed on panchromatic film in daylight with a yellow (Eastman No. K2) filter; *A*, Spotted leaves showing chlorosis and green zones around spots; *B*, young shoots infected after hail injury (from an original on Kodachrome); *C*, leafy shoots showing a prematurely ripened infected fruit (*a*), uninfected spots caused by hail injury on fruit (*b*) and leaves (*c*), and infected spots on leaves (*d*). *A*, × 1.6; *B*, × 1.2; *C*, × 0.7.

infected near the petioles fall when less spotted than leaves infected near the margins.

On petioles and tender shoots spots may occur after hail injury (fig. 2, *B*). Girdled petioles break off, or the leaves dry from lack of water and fall. Spots rarely are large enough or numerous enough to kill shoots.

On the surface of fruits small (1 mm. or less), raised, brown spots may appear. Spotted fruits are small and occasionally deformed, and they ripen prematurely (fig. 2, *C*, *a*).

At Cheyenne the spots occur principally on leaves that develop early in the season (fig. 2, *C*, *d*). Leaves that develop on new shoots late in the season usually are not affected. Infected bushes are unsightly and unthrifty, especially after severe attacks during successive seasons. Some fruits are lost because of fruit infections, and yields are probably reduced also because the plants are weakened by the loss of leaves.

ISOLATION AND PROOF OF PATHOGENICITY

The organism that causes bacterial spot was isolated first in June 1940. Portions of leaves including single typical leaf spots were surface-sterilized in a 1 : 500 solution of mercuric chloride in 50-percent ethyl alcohol (and water) for 30 seconds, washed separately in three changes of sterile tap water, and crushed in tubes of sterile tap water. They were left to diffuse in the water for 10 to 20 minutes, when a series of dilution plates (beef-extract dextrose agar) was made from each spot. Similar series of dilution plates were made from bacterial spots that followed hail injury on green shoots.

A white bacterium making moderate, opaque growth on beef-extract dextrose agar and scant, translucent growth on beef-extract agar was obtained in apparently pure culture from all spots. Few colonies of other organisms grew in any of the plates. Six isolates, including two from infected hail spots, were selected for pathogenicity tests.

Repeated inoculation tests in the field, greenhouse, and laboratory by methods described in detail elsewhere (*2*) demonstrated that all six isolates were virulently pathogenic in leaves of *Ribes aureum* but dependent on wounds or forced spray to gain access to susceptible tissues. Typical spots developed on young leaves after they were rubbed with a brush or a cloth pad wet with suspensions of the organism. Spots were more numerous on leaves that were dusted with 300-mesh carborundum before the suspensions were applied. Spots rarely developed on leaves of plants kept in moist chambers and sprayed with the suspensions. All six isolates were recovered in pure culture from inoculated leaves and found to be identical with their parent cultures, thus completing Koch's rules of proof.

DESCRIPTION OF THE PATHOGEN

ISOLATION AND PURIFICATION

Cultures of six single-colony isolates, after reisolation from inoculated currant leaves and purification by series of dilution plates, were used in preliminary cultural studies in 1942. The cultural studies

were repeated in 1943–44 with three isolates obtained by second reisolations from inoculated leaves and purification by series of dilution plates. Although mechanical methods of single-cell isolation were not used, each isolate passed through at least four single-colony transfers from dilution plates and the cultures appeared to be pure. Among several thousand colonies observed only two appeared to differ from the others. Cultures from these two were identical with the others in pathogenicity tests and in critical cultural studies in which they were compared with their parent cultures. They differed only in morphological characters as will later be shown (p. 286).

MATERIALS AND METHODS

Unless otherwise stated, the formulas and techniques used in the cultural studies were those described by the Society of American Bacteriologists (*19*). If more than one method or formula were recommended, all were used. Other formulas used are described, or the sources are cited. Except where indicated otherwise, cultures were incubated at 25° C. Growth in liquid media was compared by visual observation and by the reduction of light transmission in a Klett-Summerson photoelectric colorimeter. Hydrogen-ion concentrations of media were determined with a Beckman pH meter. Utilization of sugars was determined by the amount of growth and the change in the pH value in flask cultures with 1 percent sugar as compared with blanks and with beef-extract broth check cultures. Sugars utilized in preliminary tests with steam-sterilized media were retested twice or more in media containing sugars sterilized by filtration. Sugars not utilized in preliminary tests were retested in media containing sugars sterilized by steam. Except for potato-dextrose agar, certain synthetic media, and media used in studies on pH range, the media were adjusted to pH 7.0 before sterilization.

MORPHOLOGY AND STAINING REACTIONS

The bacterium is rod-shaped; cells occur singly, in pairs, and in hyphalike chains. Chains have cross walls difficult to distinguish even in stained mounts. The bacterium varies in size within single mounts; the dimensions of 200 cells in nonflamed, nigrosin mounts from 12-hour, beef-extract dextrose agar cultures were 0.9μ to 1.7μ long by 0.4μ to 0.9μ wide; those of 200 cells in flamed, Gram-stain mounts were 1.1μ to 1.4μ by 0.3μ to 0.6μ. Mounts from cultures of several ages grown on several kinds of media failed to show endospores or capsules. The bacterium is motile usually by one polar flagellum or under certain conditions by a tuft of two to five flagella at one end of the cell. Cells in pairs frequently have flagella at the distal end of each cell. Suspensions in tap water yield better flagella mounts than do suspensions in distilled water. The bacterium is Gram-negative and not acidfast, but it stains readily with ordinary bacterial stains.

CULTURAL CHARACTERISTICS

The bacterium is a facultative anaerobe with the temperature growth cardinals: Minimum less than 3.5° C., maximum between 30° and 32.5°, and optimum between 20° and 25°. It grows more lux-

uriantly on solid media containing 1 percent or less agar than on media with greater concentrations. Its pH tolerance is greater than the range 5.6 to 7.5; the optimum is 7.0. One hundred and sixty determinations indicated that the pH values of peptone media containing dextrose are altered in 10 days at 25° to pH 6.7 ±0.5[5] by growth of the organism. The pH value attained varies in different batches of media, particularly as a result of variation in proportions and concentrations of nutrients and amounts of inoculum. The pH values of peptone media lacking sugar are altered to pH 8.1 ±0.4.

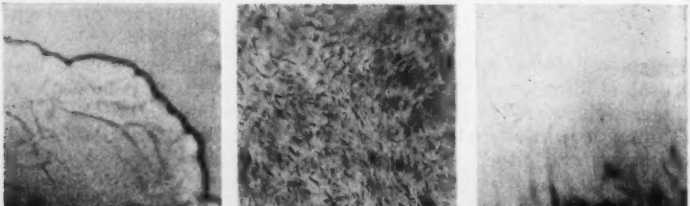

FIGURE 3.—Cultures of *Pseudomonas ribicola* on beef-extract dextrose agar in Petri dishes, photographed on panchromatic film with a green Wratten (No. 57A) filter: *A*, Lower surface of a colony showing reticulation characteristic of most isolates; *B*, upper surface at the center of a colony showing crystallike excrescences; *C*, lower surface of an atypical isolate showing curly striations. All × 80.

Beef-extract media.—On beef-extract agar 4-day-old surface colonies are punctiform, smooth, translucent, and white and have entire edges; subsurface colonies are discus-shaped. Slants yield scanty, filiform, glistening, translucent, white, slightly viscid growth. Beef-extract broth is made slightly turbid; no ring or pellicle is formed.

Beef-extract dextrose media.—On beef-extract dextrose agar 4-day-old surface colonies are circular, convex, smooth, glistening, opalescent, and white and have entire or minutely undulate edges; subsurface colonies are discus-shaped. Colonies with smooth edges have a uniform, finely granular structure; those with undulate edges have a reticulate structure near the edges. The reticulate structure is more easily observed in colonies 8 to 10 days old (fig. 3, *A*). This structural difference has not been segregated by the dilution technique of isolation and purification; it may result from differences in moisture at the agar surface. Centers of older colonies have crystallike excrescences on their surfaces (fig. 3, *B*). These are made up of hyphalike chains of cells.

Dilution plates have yielded two colonies with excessive proportions of hyphalike chains; these colonies had smooth edges and a curly striate structure (fig. 3, *C*). Cultures from these two colonies were like their parent cultures in other cultural characters and in pathogenicity, but the curly striate structure was retained after reisolation from currant leaves.

[5] Range is indicated by the ± signs in this paragraph.

Beef-extract dextrose agar slants yield moderate, filiform, glistening, opalescent white, butyrous growth. Beef-extract dextrose broth is densely clouded and made turbid; a slight ring, but no pellicle, is formed; the scanty sediment is turbid.

Potato-dextrose agar.—On potato-dextrose agar [6] 4-day-old surface colonies are circular, 6.5 mm. in diameter, convex, smooth, glistening, opalescent, and white, and their edges are entire or minutely undulate. Subsurface colonies are discus-shaped. Slants yield moderate, filiform, glistening, butyrous to slightly viscid, grayish-white growth. A dirty-pink pigment is produced in the lower parts of old cultures. The medium is slightly yellowed.

Potato plugs.—Growth on steamed potato plugs is moderate, filiform, raised, viscid, glistening, and pale yellow to cream-colored. The surface and edges are undulate.

Synthetic media.—Good growth occurs in Fermi's solution; [7] a pellicle is formed and the medium is yellowed. No growth occurs in Cohn's solution [8] or in Ashby's mannitol solution (*19*). Good growth occurs in Uschinsky's solution (*15*); a flocculent sediment is formed and the medium becomes yellowish green.

Nitrogen relations.—Milk is slightly darkened and made more alkaline; litmus is slowly reduced; slight peptonization occurs if 0.5 percent dextrose is added. Gelatin in cultures incubated at 18° C. is liquefied slowly or in occasional transfers not at all. The slight liquefaction is infundibuliform. Growth is filiform and best at the surface; the color of the medium is unchanged.

Asparagine is utilized as a sole source of carbon and nitrogen in Starr, Weiss, Klein, and Sisselman's synthetic asparagine medium (*23*).

Burkholder found this organism to be very active in oxidizing tyrosine, producing a dark-brown pigment in a medium containing this amino acid.[9]

The currant spot pathogen produces moderate growth in peptone-nitrate broth and in peptone-nitrate agar. Tests for nitrites in cultures of different ages yield erratic results; no isolate or method has given a strong positive reaction. Although growth is more abundant in such media if dextrose is added, the intensity of the reaction is not increased in carbohydrate media. Feeble growth occurs in synthetic media which contain potassium nitrate as the sole source of nitrogen. Tests for nitrites in cultures of different ages in such media also yield erratic results.

Moderate growth occurs in organic media recommended for tests for the production of ammonia, indole, and hydrogen sulfide. All tests for the production of these compounds give negative results.

Carbon relations.—The bacterium grows luxuriantly and produces moderate amounts of acids in organic liquid media containing dextrose, galactose, levulose, xylose, or mannitol. The bacterium grows moderately and produces an alkaline reaction in organic liquid media lacking carbohydrates or containing maltose, lactose, dextrin, glycogen, inulin, or starch. No gas is produced in any of these media.

The bacterium does not hydrolyze starch, but faint trace reactions occur in agar media containing this carbohydrate. Starch agar stained with iodine shows traces of red and purple coloration near the edges of streak colonies in Petri dishes.

Burkholder [10] found this organism to be inert to lipoids in spirit blue agar (*5*, *21*, *22*) and to sodium tartrate.

HABITAT

The bacterium is pathogenic on *Ribes aureum*, causing necrotic spots on leaves, tender shoots, and fruits and defoliation. It is a wound parasite in leaves, shoots, and fruits, and it probably occurs in soils.

[6] Filtrate from 200 gm. of nonpeeled boiled potato, 10 gm. of technical dextrose, 10 gm. of agar, 1 liter of distilled water.

[7] Fermi's solution: 0.2 gm. of magnesium sulfate, 1 gm. of monobasic potassium phosphate, 10 gm. of dibasic ammonium phosphate, 45 gm. of glycerol, 1 liter of distilled water.

[8] Cohn's solution: 5 gm. of monobasic potassium phosphate, 5 gm. of magnesium sulfate, 0.5 gm. of potassium chloride, 10 gm. of ammonium tartrate, 1 liter of distilled water.

[9] The formula for Starr's tyrosine medium can be obtained from Starr or from W. H. Burkholder, Cornell University, Ithaca, N. Y.

[10] Personal correspondence.

TAXONOMY OF THE PATHOGEN

The bacterium which causes necrotic spots on *Ribes aureum* is related to the green-fluorescent bacterial plant pathogens included by Bergey and others in Appendix I of *Phytomonas* (*1, pp. 169–206*). More recently this group has been included in the genus *Pseudomonas* (*4, 7, 8, 9, 22*).[11] The currant pathogen described in this paper is unlike any organism now included in that genus; hence, the name *Pseudomonas ribicola* n. sp. is proposed.

TECHNICAL DESCRIPTION

Pseudomonas ribicola n. sp.

A bacterium parasitic on *Ribes aureum* causing necrotic spots on leaves, tender shoots, and fruits. Short rod, 0.9μ to 1.7μ long by 0.4μ to 0.9μ wide in nonflamed negatively stained mounts; cells occur singly, in pairs, and hyphalike chains; organism motile by one or a tuft of two to five polar flagella; endospores or capsules not demonstrated; Gram-negative and not acidfast. Facultative anaerobe with minimum temperature for growth less than 3.5° C., maximum between 30° and 32.5°, and optimum between 20° and 25°. Grows best in culture media containing 1 percent or less agar and having an initial active acidity of pH 7.0. Colonies on beef-extract agar punctiform, translucent, white; those on beef-extract dextrose agar and potato-dextrose agar circular, convex, smooth, glistening, opalescent, white, and butyrous; internal reticulations and crystallike surface excrescences occur in dish cultures. Insoluble dirty-pink pigment produced in old tube cultures on media containing sugar. Soluble yellow or yellowish-green pigment produced in media containing sugar or asparagine. Growth on steamed potato plugs filiform, raised, glistening, pale yellow to cream-colored, and viscid. Gelatin liquefied very slowly or not at all. Good growth in beef-extract dextrose broth, Uschinsky's solution, and Fermi's solution; growth feeble in beef-extract broth, and none in Cohn's solution or Ashby's mannitol solution. Asparagine utilized as the sole source of carbon and nitrogen. Milk slightly darkened and made more alkaline; no curd or clearing; slight peptonization in dextrose milk. Inorganic nitrogen used with difficulty; nitrates reduced to nitrites weakly (erratic results); ammonia, indole, and hydrogen sulfide not produced; tyrosine oxidized. Utilizes dextrose, galactose, levulose, xylose, and mannitol, but is inert to maltose, lactose, dextrin, glycogen, inulin, and starch. Slight acid but no gas produced from utilized carbohydrates; starch not hydrolyzed. Does not oxidize lipoids or sodium tartrate.

SUMMARY

A bacterial spot found in 1939 causes severe defoliation of *Ribes aureum* in experimental, ornamental, and windbreak plantings and in plants growing naturally in the mountains in the vicinity of Cheyenne, Wyo. Necrotic spots are produced on leaves, tender shoots, and young fruits; severe attacks cause defoliation. Koch's rules of proof demonstrated that the disease is caused by a green-fluorescent, white bacterium. The morphology, staining reactions, and cultural characteristics of the parasite indicate that it is related to the green-fluorescent, white bacteria now included in the genus *Pseu-*

[11] WEISS, F., and WOOD, J. I. A LIST OF NAMES AND SYNONYMS OF PHYTOPATHOGENIC BACTERIA OCCURRING IN THE UNITED STATES (AND ITS POSSESSIONS AND A FEW OTHERS) EMBODYING RECENT CHANGES IN NOMENCLATURE. U. S. Bur. Plant Indus., Plant Dis. Rptr. 27: 42–62. 1943. [Processed.]

domonas by most students of the bacteria. The name *Pseudomonas ribicola* n. sp. is proposed and a technical description is included.

LITERATURE CITED

(1) Bergey, D. H., Breed, R. S., Murray, E. G. D., and others.
1939. bergey's manual of determinative bacteriology. Ed. 5, 1032 pp. Baltimore, Md.

(2) Bohn, G. W., and Maloit, J. C.
1945. inoculation experiments with pseudomonas ribicola. Phytopathology 35: 1008–1016, illus.

(3) Bondartzev, A. S., and Serbinov, I. L.
1913. [diseases of small fruits and vegetables and their control. part 2, diseases of fruits and vegetables.] 108 pp., illus. St. Petersburg. [In Russian.]

(4) Burkholder, W. H.
1939. the taxonomy and nomenclature of the phytopathogenic bacteria. Phytopathology 29: 128–136.

(5) Castell, C. H.
1941. p-aminodimethylaniline monohydrochloride as an indicator of microbial action on fats. Stain Technol. 16: 33–36.

(6) Dennis, R. W. G., and Foister, C. E.
1942. list of diseases of economic plants recorded in scotland. Brit. Mycol. Soc. Trans. 25: 266–306, illus.

(7) Dowson, W. J.
1941. on the systematics of gram negative bacterial plant pathogens. Chron. Bot. 6: 198–199.

(8) ———
1943. on the generic names pseudomonas, xanthomonas and bacterium for certain bacterial plant pathogens. Brit. Mycol. Soc. Trans. 26: 4–14.

(9) Elliott, C.
1943. recent developments in the classification of bacterial plant pathogens. Bot. Rev. 9: 655–666.

(10) [Great Britain] Ministry of Agriculture and Fisheries.
1932. collected leaflets on fungus and allied diseases of fruit trees. Ed. 6, Sect. Vol. 1, 83 pp., illus. London.

(11) Hesler, L. R., and Whetzel, H. H.
1917. manual of fruit diseases. 462 pp., illus. New York.

(12) Hildebrand, E. M.
1942. tomato ringspot on currant. Amer. Jour. Bot. 29: 362–366, illus.

(13) Kirchner, O. von.
1923. die krankheiten und beschädigungen unserer landwirtschaftlichen kulturpflanzen. Ed. 3, 679 pp. Stuttgart.

(14) Lochhead, W.
1904. insects and fungus diseases. Ontario Agr. Col. and Expt. Farm Ann. Rpt. 30: 40–49, illus.

(15) McLean, R. C., and Cook, W. R. I.
1941. plant science formulae; a reference book . . . 203 pp. London.

(16) Paine, S. G.
1918. an epitome of bacterial diseases of plants in great britain and ireland. Ann. Appl. Biol. 5: 62–76.

(17) Severin, H. C.
1920. the most important insect enemies and plant diseases attacking the leaves of currants and gooseberries. S. Dak. State Hort. Soc. Ann. Rpt. 17: 191–206, illus.

(18) Smith, E. F.
1920. an introduction to bacterial diseases of plants. 688 pp., illus. Philadelphia and London.

(19) SOCIETY OF AMERICAN BACTERIOLOGISTS, COMMITTEE ON BACTERIOLOGICAL TECHNIC.
1941–43. MANUAL OF METHODS FOR PURE CULTURE STUDY OF BACTERIA. Ed. 8. Leaflets I, II, IV, V, IX. Geneva, N. Y.

(20) STAPP, C.
1927. DER BAKTERIELLE PFLANZENKREBS UND SEINE BEZIEHUNGEN ZUM TIERISCHEN UND MENSCHLICHEN KREBS. Deut. Bot. Gesell. Ber. 45: 480–504. [Abstract in Biol. Abs. 3: 17652. 1929.]

(21) STARR, M. P.
1941. SPIRIT BLUE AGAR: A MEDIUM FOR THE DETECTION OF LIPOLYTIC MICROORGANISMS. Science 93: 333–334.

(22) ——— and BURKHOLDER, W. H.
1942. LIPOLYTIC ACTIVITY OF PHYTOPATHOGENIC BACTERIA DETERMINED BY MEANS OF SPIRIT BLUE AGAR AND ITS TAXONOMIC SIGNIFICANCE. Phytopathology 32: 598–604.

(23) ——— WEISS, J. E., KLEIN, H. P., and SISSELMAN, C. B.
1943. GROWTH OF PHYTOPATHOGENIC BACTERIA IN A SYNTHETIC ASPARAGIN MEDIUM. Phytopathology 33: 314–318.

HAPLOIDY IN EINKORN [1]

By LUTHER SMITH

Associate geneticist, Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture

INTRODUCTION

The study of haploids and haploidy has been of considerable interest from a practical standpoint because of the possibility that completely homozygous inbred lines could be developed rapidly by deriving diploids from haploids through chromosome doubling. The subject has also been of interest because of the theoretical implications involved in the development of higher organisms with a complete soma made up of cells containing only the gametic number of chromosomes. Furthermore, many studies have been made on haploids for the purpose of gaining information on the quantitative effects of genes and on the physical (cytological) mechanism of inheritance.

The present paper presents observations on the occurrence of haploids in untreated populations of einkorn (*Triticum monococcum* L.), among F_1 hybrids, after X-radiation of pollen, from delaying pollination, and among twin seedlings. Observations on the meiosis, secondary pairing, fertility, and progeny of haploids of einkorn are included also.

REVIEW OF LITERATURE

Some of the many papers bearing on different phases of the present study will be mentioned in connection with the various topics, but more complete reviews of the literature and additional citations, together with an analysis of the factors that affect the occurrence of haploids, may be found in the excellent papers by Ivanov (*6*),[2] Kostoff (*16*), and Bleier (*1*) and in a bibliography of the Imperial Bureau of Plant Genetics (*5*).

Haploids have been reported in about 20 genera of plants. In only a few of these genera have they occurred in sufficient numbers for information on frequencies. Lethal factors probably materially reduce the number of observed haploids in cross-pollinated species (*18*). Haploids in maize occur at the rate of about 1 in 2,000 plants, according to Randolph and Stadler.[3] Satina, Blakeslee, and Avery (*20*) reported observing 73 haploids among approximately 410,000 *Datura* plants (0.018 percent). Harland (*4*) reported a rate of 1 haploid among 3,000 to 4,000 plants of sea-island cotton. Jones and Longley (*8*) found 11 haploidlike plants among about 300,000 rice plants.

[1] Received for publication September 22, 1944. This work was carried out in cooperation with the Missouri Agricultural Experiment Station. Contribution from the Field Crops Department, Missouri Agricultural Experiment Station. Journal Series Paper No. 933.

[2] Italic numbers in parentheses refer to Literature Cited, p 300.

[3] From unpublished data by L. F. Randolph and L. J. Stadler.

Johansen (*7*) reported an unusual frequency (about 10 percent) of haploids from 1 commercial lot of barley.

Comparative figures are not available, but from the number of reported cases it would appear that haploids occur with a higher frequency in einkorn than in emmer or common wheat. Yamasaki (*24*) found 48 haploids among an undisclosed number of diploids of common wheat. Katayama (*10*, *11*) reported a frequency of 0.48 percent of haploids in ordinary field cultures of 1 variety of einkorn, and 4.9 percent in certain progenies 2 and 3 generations after irradiation. Smith (*21*) reported a frequency of over 20 percent of haploids in the progeny of 1 plant of einkorn.

MATERIAL AND METHODS

The populations studied and reported in this paper were almost entirely from varieties and derived strains of the species *Triticum monococcum*, but some lines were derived from crosses of *T. monococcum* with *T. aegilopoides* (Link) Bal.

To obtain irradiated pollen, spikes of the pollen parent were placed 12 inches from the tube's target and treated at about 140 kilovolts (peak), 4 milliamperes (ma.) tube current, shortly before the pollen was applied to previously emasculated spikes. The acetocarmine smear technique was used in studying microsporogenesis. Carnoy's solution was used as a fixative.

EINKORN HAPLOIDS

DESCRIPTION

Haploids have been found and described in einkorn and in so many related species and genera that only a few observations concerning the morphology of those reported in this paper seem necessary. Forty-two haploids averaged 23.5 inches in height, as compared with 36.5 inches for 183 diploid sibs, or about two-thirds of the latter. The average number of tillers was 12.0 and 12.4, respectively. The leaves of haploids were narrower and shorter, and the stomates and spikes were smaller. With a little experience the observer can readily detect haploids at heading, or later, by their generally more delicate appearance.

OCCURRENCE IN VARIOUS POPULATIONS

Untreated Populations

Among 8,700 individuals observed in 1941 and 1942, there were 7 haploids (0.08 percent). One plant of *Triticum monococcum* var. *hornemanni* (Clem. y Rubio) Koern. and Wern. grown in the greenhouse in 1932–33 gave rise to 9 haploids among 39 offspring (23 percent). Other individuals of this variety showed no unusual tendency to produce haploid progeny.

F_1 Hybrid Populations

Many of the haploids reported in the literature were from species hybrids or other crosses, and crossing is one of the methods most frequently suggested for obtaining haploids. However, Satina, Blakeslee, and Avery (*20*) reported that haploids in *Datura* were no more frequent from interspecific and intraspecific crosses than from selfs.

There is no doubt that, in the present studies, haploids have occurred more frequently in populations of F_1 hybrids of einkorn than in normally self-pollinated progenies. Table 1 presents data on the number of haploids in various stocks of einkorn, classified according to the female parent. A number of pollen parents were used on each stock. Thus, there were 49 haploids among 2,478 hybrids, as compared with 7 among 8,700 individuals from naturally self-pollinated populations.

TABLE 1.—*Haploids in various F_1 hybrid populations of einkorn*

Kind of female parent	Total population	Diploid plants	Haploid plants	
	Number	*Number*	*Number*	*Percent*
Normal	990	973	17	1.7
Male-sterile	1,121	1,103	18	1.6
Fragments [1]	117	114	3	2.6
Deficiency-duplication [2]	[3] 250	232	11	4.4
Total	2,478	2,422	49	2.0

[1] In the fragments stock, 1 chromosome had been broken, presumably by X-rays, and was represented by 2 fragments.
[2] The deficiency-duplication stock had a viable deficiency and duplication complex resulting from the functioning of gametes produced by nondisjunctional segregation of a reciprocal translocation.
[3] There were also 7 triploids (2.8 percent) in these progenies.

The "deficiency-duplication" stock gave an especially high frequency of haploids. Among these same crosses with this stock there was also a high percentage (2.8) of triploids. All the triploids had the deficiency-duplication complex. It would be of interest to know whether the frequency of haploids and triploids in this stock is referable to the mechanism which produces haploid-triploid twin seedlings.

In every instance (at least 14) where the pollen and egg parents could be distinguished, the haploid exhibited the characteristics of the maternal plant. No experiment was set up and no special effort was made to detect maternal diploids. The presence of male-sterile diploids among F_1 populations of male-sterile plants pollinated with pollen from normal individuals indicated that maternal diploids do occur.

Populations From X-rayed Pollen

Kihara (*12*) summarized the results obtained by Kihara and Katayama (*13*), Katayama (*9*), and Kihara and Yamashita (*14*) on the production of haploids in einkorn by means of X-rayed pollen. Among 138 individuals they found 19 haploids (13.8 percent). Ivanov (*6*) obtained 1 haploid of *Nicotiana rustica* var. *humilis* by pollinating untreated flowers with heavily X-rayed pollen (17,000 to 30,000 roentgens (r.)) of the variety *texana* and 3 haploids from the reciprocal cross. These 4 haploids occurred among about 336 plants that grew to the end of the vegetative period. Ivanov believed that X-raying the pollen produced a significant increase in the number of haploids. He (*6*) and Kostoff (*16*) also cited a few other instances of successful use of X-rays in producing haploids.

The writer observed no effect of X-rays on the production of haploids in einkorn. Among 207 individuals obtained by pollinating untreated spikes with pollen given a dose of 520 r. (14 were given only 260 r.), only 1 was haploid. This was a somewhat lower fre-

quency than in F_1 populations when untreated pollen was used. The dose was not heavy; this may account for the failure of the treatment to increase the number of haploids. However, a dose of 1,000 r. applied to pollen of einkorn considerably reduced seed production. Sears[4] found that 2,000 r. applied to pollen of emmer and common wheat materially reduced both seed set and germination. He found no haploid among a small number of plants grown from seeds resulting from the treated pollen.

Also, the writer obtained no haploids as a result of X-raying pollen of tetraploid or hexaploid wheat or of an einkorn-*Aegilops uniaristata* Vis. amphidiploid ($2n=28$). The dose applied was 520 r. and the populations examined numbered 264, 235, and 144 individuals, respectively.

Randolph[5] found a slight increase in the frequency of haploids when irradiated pollen was applied to untreated ears of maize. He observed a frequency of 1 haploid to 1,550 diploids from untreated pollen as compared with 1 to 1,040 from X-rayed pollen (1,500 r.). These frequencies were based on populations of 171,000 and 84,000 plants, respectively. The female parent stock had a greater influence than X-rays on the frequency of haploids.

Populations From Delayed Pollinations

To the factors suspected or known to affect the production of haploids (hybridization, polyembryony, genetic constitution, heat, cold, X-radiation) has been added delayed pollination. Kihara (*12*) reported that if pollination of einkorn wheat was delayed beyond the normal time after emasculation the frequency of haploids increased markedly with time. He found no haploid among 41 individuals from pollinations 2 to 5 days after emasculation. Pollinations on the sixth day gave 2 haploids among 10 plants; on the seventh day, 4 haploids among 44 plants; on the eighth day, 5 haploids among 18 plants; and on the ninth day, 3 haploids among 8 plants.

Table 2.—*Effect of delayed pollination on the production of haploids in a male-sterile einkorn*

Days between opening of first florets and pollination	Spikes pollinated	Germination	Progeny			
			Total plants	Diploid plants	Haploid plants	
	Number	*Percent*	*Number*	*Number*	*Number*	*Percent*
3	17	90	188	186	2	1.1
6	17	89	202	191	11	5.4
9	24	78	63	50	13	20.6

Kihara's finding was confirmed by the data of table 2. A male-sterile einkorn was used as the female parent in these crosses, thus avoiding the necessity of emasculating. When the first florets of spikes on the male-sterile plant opened, the spikelets were clipped off with scissors, as is done when they are emasculated. The clipped spikes were covered with parchment bags, and pollen from various male parent stocks was applied 3, 6, and 9 days after the clipping was done. The period between the opening of the first and last florets

[4] From unpublished observations by E. R. Sears.

[5] From unpublished data by L. F. Randolph, which he has kindly permitted the writer to mention.

probably would have been 3 or 4 days. If emasculation had been necessary, it would have been done at least 3 days before the spikelets were clipped. Thus the times of pollination would correspond to about 6, 9, and 12 days after emasculation. Some spikes not pollinated for 12 days set no seed at all.

Twin Seedlings

Twin seedlings are a source of haploids, as shown by the studies of Müntzing (*17*) and others. Kihara (*12*) reported that twin seedlings were never found in the progeny of *Triticum monococcum* from self- or open-pollinations but were rather numerous (0.78 percent) from cross-pollinations. In a delayed-pollination experiment he observed 3 pairs of twins (chromosome number not reported) among 195 seedlings.

The writer has not kept a complete record of the occurrence of twin seedlings in einkorn, but they have been rare in both naturally self- and cross-pollinated progenies, delayed or otherwise. In one experiment no twin was found among 2,855 seedlings from naturally self-pollinated spikes. The writer has not determined the chromosome number of twin seedlings.

Meiosis

Katayama (*10*) made a rather thorough study of meiosis in haploids of einkorn. He reported that of 500 pollen mother cells 10 had 1 "bipartite" chromosome at first metaphase. The writer found 73 bivalents in 2,276 cells (3.2 percent) from 12 haploid plants. Not a single cell with a closed bivalent or more than 1 bivalent was observed among these and a large number of additional cells which were not tabulated. Yamasaki (*24*) observed that 1.2 percent of the bivalents in a haploid of common hexaploid wheat were closed bivalents, indicating that there is considerable homology between some of the chromosomes.

It is problematical whether pairing at metaphase in wheat haploids is indicative of true homology. Kostoff (*15*) was of the opinion that, in haploid einkorn, pairing is between inert, heterochromatic regions and has nothing to do with homology. However, in some of the bivalents in haploid einkorn the attachment appears quite strong, and close examination indicates that there are connections between the threads of the two chromosomes involved. In some bivalents there is a definite indication of a chiasma, as shown in figure 1, *A*. If pairing were nonspecific, as Kostoff suggests, it would seem that two or even three pairs might have been observed. Also, closed bivalents presumably would be expected to occur.

Occasionally a bridge or a bridge and fragment were observed at first or second anaphase or telophase (fig. 1, *B* and *D*). Such a bridge has been described in haploids of other species, and a bridge with a large fragment was observed in a haploid of einkorn by Katayama (*10*). An effort was made in the present study to compare the frequency of bridges with the frequency of bivalents. At early first telophase, 9 of 309 cells (2.9 percent) had a bridge and in 8 of the 9 a small fragment was visible. At second anaphase, 1 of 10 cells, and, at early second telophase, 1 of 33 cells had a bridge but no visible fragment. The fragment observed at first telophase was tiny in each case but there seemed to be a slight variation in the size of the fragments. These observations show that the frequency of bridges is

comparable to the frequency of bivalents. However, apparently not all bivalents result in bridges (fig. 1, *C*).

Bridges or bridges and fragments of the type described usually have been interpreted as the result of crossing over between struc-

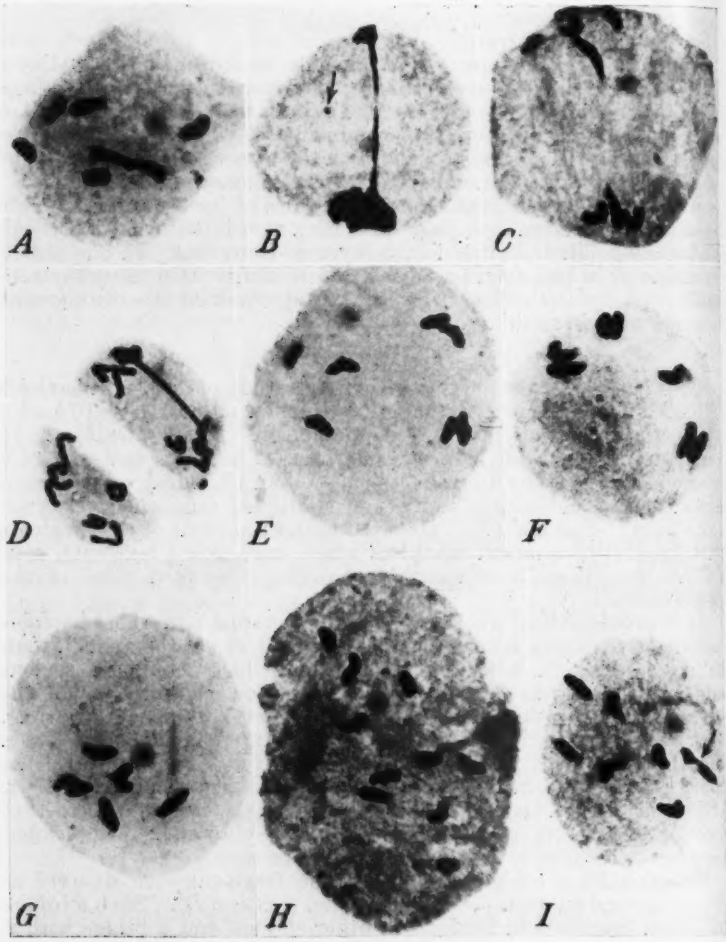

FIGURE 1.—*A*, A bipartite (bivalent), apparently with a subterminal chiasma; a considerable portion of the bivalents in haploids of einkorn seem to be closely attached. *B*, A bridge and small fragment (see arrow) at early first telophase. *C*, First anaphase in a cell in which 2 chromosomes were apparently attached at metaphase but are separating without forming a bridge. *D*, A bridge at second anaphase. *E*, A cell with 2 secondary pairs. (See text.) *F*, A cell with 3 secondary pairs. *G*, A cell with only 6 chromosomes; cells with other than 7 chromosomes were rare, but cells with 5, 6, 8, 10, and 14 chromosomes were observed. *H*, A cell with 10 chromosomes. *I*, A cell with 1 chromosome (see arrow) apparently undergoing breakage or misdivision. × 850.

turally dissimilar chromosomes. If this interpretation is applicable to the bridges in haploid einkorn, it would suggest that crossing over had occurred between most of the bivalents. The bridges at second anaphase and telophase could result from the failure of the attached chromatids to separate in the first division.

SECONDARY PAIRING AND THE BASIC NUMBER OF CHROMOSOMES IN WHEAT

Admittedly, a discussion of the basic number of chromosomes in any species or genus is more or less fruitless because, as pointed out by Tometorp *(23, pp. 252–253)*, "The basic number is one . . .," and if this reasoning is pursued somewhat further, a subdivision of the chromosome, i. e. the gene, is basic. However, the basic number of chromosomes is a topic of considerable interest in connection with studies of phylogeny.

Pathak's (*19*) observation of occasional quadrivalents and secondary associations in *Triticum monococcum*, together with other considerations, led him to conclude that five is the basic number among all the grasses. Haploids in einkorn should furnish especially favorable material for a consideration of the basic number of chromosomes in wheat, particularly as judged by secondary pairing. It should be pointed out, as Flovik (*3*) and others have done, that there are many who doubt that secondary pairing has any significance in indicating homologies or homeologies between chromosomes. However, if it is assumed that secondary pairing occurs and that it indicates affinity between parts of chromosomes that do not ordinarily enter into a primary, bivalent, association, then in haploids, where normal pairing cannot occur, the opportunity for secondary pairing would seem to be especially favorable. If this is true and five is taken as the basic number of chromosomes in einkorn, two associations of a secondary character, each involving two chromosomes, would presumably occur more frequently in a haploid than would be expected if the univalents were distributed at random.

Tabulation of the arrangements of chromosomes in the pollen mother cells on slides from four haploid plants gave the following results:

Kind and distribution of chromosome groups:	*Number of pollen mother cells*
Secondary pairs: [1]	
0	24
1	89
2	90
3	25
Groups having more than 2 chromosomes: [2]	
3	62
4	38
5	22
6	10
7	0
Two and three groups having 2 or more chromosomes:	
3–2	95
3–3	8
3–4	2
4–2	14
5–2	3
3–2–2	11

[1] Two chromosomes were considered paired if they were not more than the diameter of a chromosome apart at some one point (fig. 1, *E* and *F*). Only a small percentage were paired throughout their length. Rod bivalents comparable to those in figure 1, *A*, were excluded.

[2] A group was made up of chromosomes at least 2 of which, but not necessarily all, were close enough to be considered paired in the sense of footnote 1.

Certainly smearing distorted the pollen mother cells and altered the relations of the chromosomes from what they were in living cells, but presumably this error was random and does not affect the interpretation of results.

The evidence indicated that there was a tendency for two chromosomes to be near two other chromosomes in the pollen mother cells. Perhaps the strongest evidence for this conclusion was in the observation that if a group of three was present in a cell there was usually another group of two, a pair, present in the same cell, but very rarely was another group of three present. However, the predominant arrangement of the chromosomes was by no means two secondary pairs.

In the hypothesis of secondary pairing it is assumed that homology between the chromosomes is insufficient to bring about, or that structural changes hinder, primary association. If primary association were prevented by a known structural change, secondary association between the chromosomes would presumably still occur. It is outside the scope of the present study to go into detail regarding the hypothesis of secondary pairing, but it might be of interest to mention some observations on the arrangements of chromosomes where primary association was prevented, in one instance by a translocation and in another instance by an inversion.

Smith (*22*) reported a translocation in barley that not infrequently formed 7 pairs instead of 5 pairs, plus a ring or chain of 4 chromosomes. Even though a ring or chain was not formed, if secondary pairing was exerting a strong influence, the 2 pairs of chromosomes (distinguished by being open or rod-shaped) should lie close together in the cell at metaphase. The results tabulated from a limited number of cells (46) showed that if there was any such tendency it was slight.

Similar observations on the distribution of 2 univalents in einkorn, prevented from primary association by an inversion, showed even less tendency for the 2 univalents to be near one another than was the case with the bivalents from the translocation. The arrangements of the univalents were tabulated in 57 cells.

Although the foregoing observations on the translocation and inversion do not disprove the possibility that secondary pairing may take place, they do not tend to support that hypothesis.

FERTILITY

Katayama (*10*) found 20 seeds in 8,285 spikelets on 289 spikes of haploid plants of einkorn.

The writer did not keep a complete record of the fertility of haploids, but some observations may be of interest. Under field conditions, each haploid plant on the average had 1 seed. Quite a number had 3 to 5 seeds. The largest number of seeds produced by a single haploid plant was 21. A very few others had 10 or more. No haploid grown in the greenhouse ever produced a seed, although the number grown to maturity in the greenhouse was a considerable fraction of the total.

O'Mara [6] obtained no seed on about 150 bagged spikes of haploids, although unbagged spikes did set seed. This number of spikes is not

[6] From unpublished data by J. G. O'Mara.

large, but, together with the absence of seeds on greenhouse plants, it suggests that the production of seeds is associated with, or conditioned by, cross-pollination, which is much more likely to occur under outdoor conditions.

PROGENY

Katayama (*10*) reported that of 6 plants grown from seeds of haploid individuals 3 were diploid and 3 haploid. The diploid offspring had 7 bivalents with about the same number of chiasmata as diploids from diploid parents. The writer, O'Mara, and Sears [7] grew 90 plants of einkorn from haploid parents. Of these 2 were haploid and the rest diploid. Of the 88 diploids, 30 were examined cytologically at meiosis; 17 among the 30 had a ring of 4 plus 5 bivalents instead of 7 bivalents. However, all these 30 plants had come from stocks which had at one time been subjected to irradiation. It is a matter for speculation whether these rings arose from irradiation or were causally related to "pairing" in the haploid female parents.

Instances in which haploid plants have developed diploid chimeras are numerous, and there is little doubt that diploid sectors occur in haploid einkorn plants, although they are not frequent. Among the several score haploids the writer has observed, there were only two or three almost certain instances of diploid sectors that resulted in seed production.

DISCUSSION

In most reported instances haploids developed parthenogenetically, and only a few instances of androgenesis are known. Therefore, any information on parthenogenesis is of value in interpreting the origin of haploids. In this connection, Cooper (*2*) observed a number of instances in *Lilium* and *Nicotiana* in which haploid embryos (particularly among twins) arose by proliferation of a synergid, a phenomenon previously postulated on genetical grounds. Kihara (*12*) referred to a study by Katayama in which the latter observed many-celled embryos in ovaries of wheat spikes that had not been pollinated for 9 days after emasculation. As Kihara and the writer have shown, if these spikes with many-celled embryos had been pollinated, some seeds would have developed and some of these seeds probably would have produced haploids.

There is insufficient evidence to determine whether these observations of Cooper and Katayama explain the origin of all apogamous haploids. If they do, the more frequent occurrence of haploids among populations resulting from crosses (particularly wide crosses), twin seedlings, delayed pollinations, and possibly irradiation and temperature variations is explicable. It would seem probable that in the present study the increase in the number of haploids in intraspecific hybrid populations was due to the delayed-pollination effect—that the haploid embryo had already begun to develop, and when pollination provided an endosperm, a seed with a haploid embryo developed. In wide crosses possibly the endosperm with two maternal and one paternal set of chromosomes is capable of developing; but the embryo with one maternal and one paternal set dies, in which event a synergid, or an already partially developed maternal embryo, develops into a haploid embryo. Phenomena similar to those postulated may account for haploids from twin seedlings.

[7] From unpublished data by L. Smith, J. G. O'Mara, and E. R. Sears.

Whether or not the data presented are taken to indicate that the basic number of chromosomes in wheat is five depends on the personal evaluation of the significance of secondary pairing. Those who believe in secondary pairing will form a different opinion from those who think that secondary pairing is an artifact or that it results from cytoplasmic factors independent of homologies between the chromosomes.

The results obtained confirm Kihara's (*12*) finding that it is possible to obtain a considerable percentage of haploids of einkorn by delaying pollination. If this simple expedient, or an adaptation of it, were equally effective in other species, delaying pollination and the colchicine technique for chromosome doubling would make possible the production in abundance of homozygous inbred lines for agronomic and theoretical uses. Unfortunately, according to Stadler,[8] simply delaying pollination has not proved very successful in the production of haploids in maize.

SUMMARY

In the present study of einkorn (*Triticum monococcum*) haploids were found to occur in untreated populations at the rate of about 1 per 1,000, whereas about 20 haploids per 1,000 occurred among intraspecific hybrids and up to 200 per 1,000 from delayed pollinations. Moderate doses of X-rays applied to pollen did not appreciably increase the frequency of haploids.

Approximately 3 percent of the pollen mother cells of haploids had one rod bivalent at first metaphase, and about the same percentage had a bridge or a bridge and small fragment at first telophase.

Four chromosomes were frequently arranged at first metaphase so that they might be considered as forming two secondary pairs. This could be taken as evidence that the basic number of chromosomes in wheat is five. Some consideration was given to the significance of secondary pairing.

Haploids produced few seeds, and these probably from cross-pollination. Almost all the offspring were diploids.

LITERATURE CITED

(1) Bleier, H.
1933. Die meiosis von haplodiplonten. Genetica 15: 129–176, illus.

(2) Cooper, D. C.
1943. Haploid-diploid twin embryos in lilium and nicotiana. Amer. Jour. Bot. 30: 408–413, illus.

(3) Flovik, K.
1938. Cytological studies of arctic grasses. Hereditas 24: 265–376, illus.

(4) Harland, S. C.
1936. Haploids in polyembryonic seeds of sea island cotton. Jour. Hered. 27: 229–231.

(5) Imperial Bureau of Plant Genetics (for Crops Other Than Herbage).
1936. The experimental production of haploids and polyploids. 27 pp. Cambridge, England.

(6) Ivanov, M. A.
1938. Experimental production of haploids in nicotiana rustica L. (and a discussion of haploidy in flowering plants). Genetica 20: 295–397, illus.

[8] From unpublished data by L. J. Stadler.

(7) JOHANSEN, D. A.
1934. HAPLOIDS IN HORDEUM VULGARE. Natl. Acad. Sci. Proc. 20: 98–100, illus.
(8) JONES, J. W., and LONGLEY, A. E.
1941. STERILITY AND ABERRANT CHROMOSOME NUMBERS IN CALORO AND OTHER VARIETIES OF RICE. Jour. Agr. Res. 62: 381–399, illus.
(9) KATAYAMA, Y.
1934. HAPLOID FORMATION BY X-RAYS IN TRITICUM MONOCOCCUM. Cytologia 5: 235–237, illus.
(10) ———
1935. KARYOLOGICAL COMPARISONS OF HAPLOID PLANTS FROM OCTOPLOID AEGILOTRICUM AND DIPLOID WHEAT. Jap. Jour. Bot. 7: 349–380, illus.
(11) ———
1935. KARYOGENETIC STUDIES ON X-RAYED SEX CELLS AND THEIR DERIVATIVES IN TRITICUM MONOCOCCUM. Tokyo Imp. Univ., Col. Agr. Jour. 13: [333]–362, illus.
(12) KIHARA, H.
1940. FORMATION OF HAPLOIDS BY MEANS OF DELAYED POLLINATION IN TRITICUM MONOCOCCUM. Bot. Mag. [Tokyo] 54: 178–185, illus. [In Japanese. English summary, pp. 183–185.]
(13) ——— and KATAYAMA, Y.
1932. ON THE PROGENY OF HAPLOID PLANTS OF TRITICUM MONOCOCCUM. Kwagau 2: 408–410. [In Japanese, with German summary.]
(14) ——— and YAMASHITA, K.
1939. ARTIFICIAL PRODUCTION OF HAPLOID AND TRIPLOID EINKORN WHEATS BY POLLINATION WITH X-RAY IRRADIATED POLLEN. (Commemoration papers on agronomy prepared in honour of Professor Masao Akemine on the occasion of the thirtieth anniversary of his academic service by his friends and pupils, April 1938: 9–20.) Imp. Bur. Plant Breeding and Genet. Plant Breeding Abs. 9: 298.
(15) KOSTOFF, D.
1938. HETEROCHROMATIN AT THE DISTAL ENDS OF THE CHROMOSOMES IN TRITICUM MONOCOCCUM. Nature [London] 141: 690–691, illus.
(16) ———
1941. THE PROBLEM OF HAPLOIDY. (CYTOGENETIC STUDIES ON NICOTIANA HAPLOIDS AND THEIR BEARINGS TO SOME OTHER CYTOGENETIC PROBLEMS.) Bibliog. Genet. 13: 1–148, illus.
(17) MÜNTZING, A.
1938. NOTE ON HETEROPLOID TWIN PLANTS FROM ELEVEN GENERA. Hereditas 24: 487–491.
(18) NORDENSKIÖLD, H.
1939. STUDIES OF A HAPLOID RYE PLANT. Hereditas 25: 204–210, illus.
(19) PATHAK, G. N.
1940. STUDIES IN THE CYTOLOGY OF CEREALS. Jour. Genet. 39: 437–467, illus.
(20) SATINA, S., BLAKESLEE, A. F., and AVERY, A. G.
1937. BALANCED AND UNBALANCED HAPLOIDS IN DATURA. Jour. Hered. 28: 193–202, illus.
(21) SMITH, L.
1936. CYTOGENETIC STUDIES IN TRITICUM MONOCOCCUM L. AND T. AEGILOPOIDES BAL. Mo. Agr. Expt. Sta. Res. Bul. 248, 38 pp., illus.
(22) ———
1941. AN INVERSION, A RECIPROCAL TRANSLOCATION, TRISOMICS, AND TETRAPLOIDS IN BARLEY. Jour. Agr. Res. 63: 741–750, illus.
(23) TOMETORP, G.
1939. CYTOLOGICAL STUDIES ON HAPLOID HORDEUM DISTICHUM. Hereditas 25: [241]–254, illus.
(24) YAMASAKI, Y.
1936. SOME OBSERVATIONS ON THE MICROSPOROGENESIS OF THE HAPLOID PLANT OF TRITICUM VULGARE HOST. Jap. Jour. Bot. 8: 151–153, illus.

U. S. GOVERNMENT PRINTING OFFICE: 1946